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SPERMATOGENIC REBOUND PHENOMENON AFTER ADMINISTRATION OF TESTOSTERONE PROPIONATE*

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IN 1939, it was observed and reported by one of us (1) that an azoospermia developed in a man who was treated with testosterone propionate. It was further noted that, when the administration of the androgen was discontinued, there was a gradual return of spermatogenesis, which reached not only the pretreatment level but far exceeded it.

Heller, Nelson, Hill, Henderson, Maddock, and Jungck (2) have recently made an admirable study on the effect of testosterone administration upon the testes of androgenically normal men. They obtained testicular biopsy specimens before administration of testosterone, at the end of treatment, and six months to seventeen months after treatment. They found that after administration of a course of testosterone propionate there was a complete disappearance of Leydig cells, marked decrease in the size of the seminiferous tubules, arrest of spermatogenesis or necrosis of the germinal elements, and marked hyalinization of the basement membrane and tunica propria of the tubules. Biopsy specimens taken six months and seventeen months after cessation of treatment showed re-

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covery and, in 5 cases, the testicular structure was appreciably better than in the initial specimens.

This report records our observations on the effect of testosterone propionate[1] in 5 patients who had a moderate oligospermia of undetermined

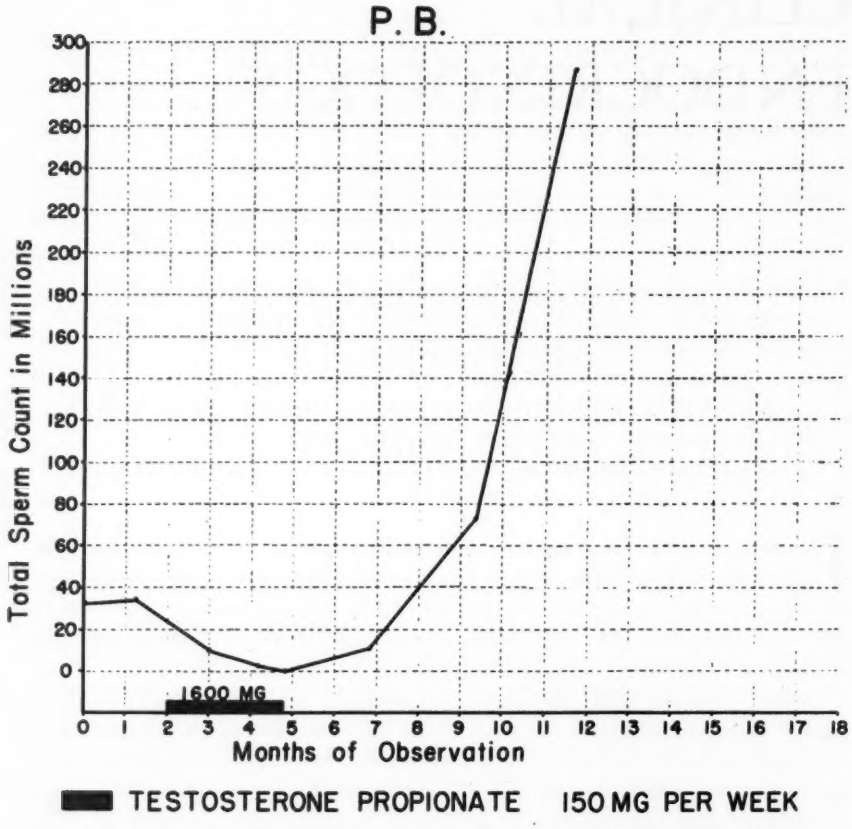

FIG. 1. *P. B., Case 1.* Showing marked increase in number of spermatozoa after initial azoospermia produced by 1,600 mg. of testosterone propionate.

etiology. They were first made azoospermic or nearly so by the intramuscular administration of 50 milligrams of testosterone propionate three times a week. Analyses of the seminal fluid were made at six-week to eight-week intervals for as long as twenty-nine weeks after treatment was dis-

[1] The testosterone propionate used in this study was supplied by the Schering Corporation, Bloomfield, N. J.

continued. Testicular biopsies were performed on the 5 patients before treatment was started; and on 3 of the patients a second biopsy was performed seventeen weeks, twenty weeks, and twenty-two weeks respectively after therapy was stopped.

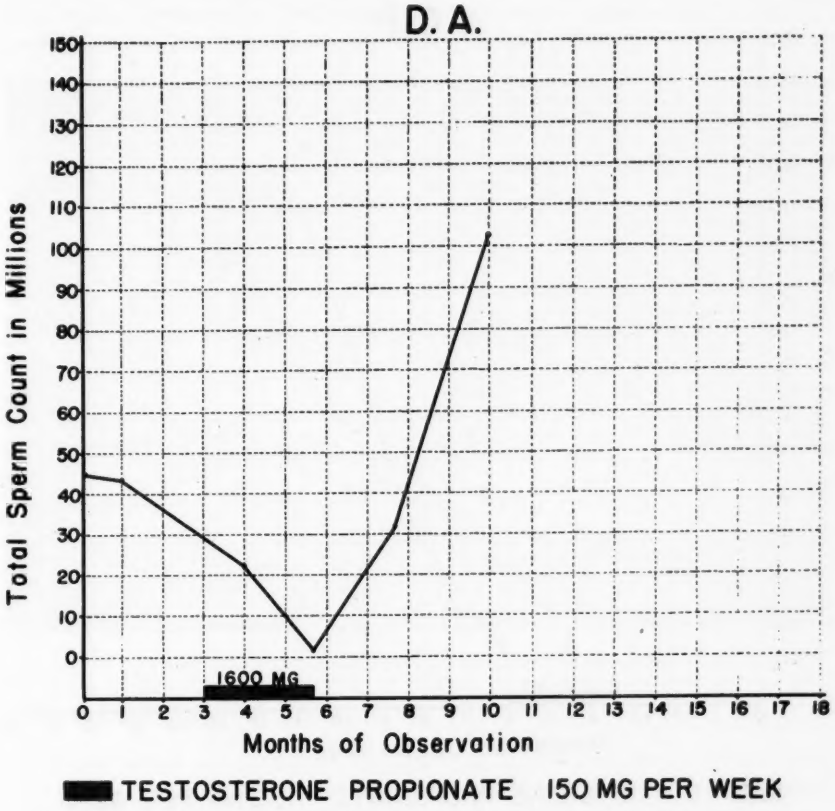

FIG. 2. *D. A., Case 2.* Marked increase in spermatogenic activity following near-azoospermia produced by 1,600 mg. of testosterone propionate.

CASE REPORTS

Case 1. P. B., aged 34, had a sterile marriage of fourteen years' duration. The past and family histories were irrelevant, as was the sexual history.

He was normally developed and potent. His weight was 171 pounds, and height 66 inches. The testes, epididymii, prostate gland, and prostatic fluid were normal. There were no unusual findings in the routine laboratory tests. Two basal metabolic rates were within normal limits. Several seminal fluid examinations (Fig. 1) revealed a moderate

oligospermia of 31 million.[2] A testicular biopsy showed a moderate thickening of the basement membrane of the seminiferous tubules. Spermatogenesis appeared to be arrested at the spermatocyte stage in about 50 per cent of the tubules. There was a slight decrease in the number of interstitial cells.

The patient was given 50 milligrams of testosterone propionate intramuscularly three

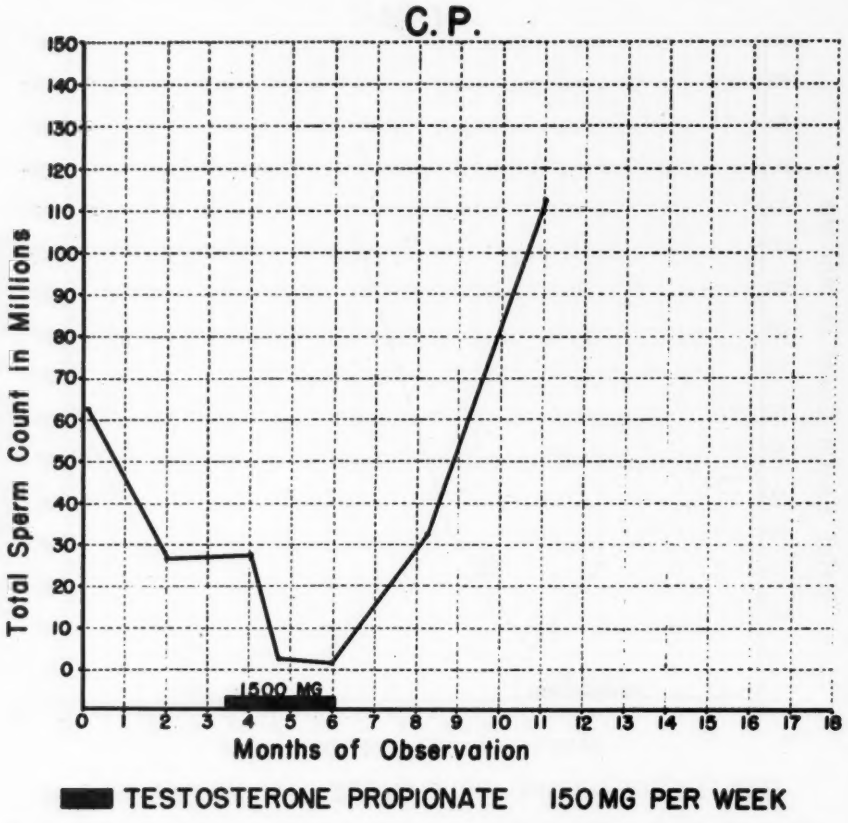

FIG. 3. *C. P., Case 3.* Near-azoospermia during administration of testosterone propionate, followed by sharp rise in sperm count after discontinuance of treatment.

times a week. After he had received 1,600 milligrams over a twelve-week period, he was azoospermic and treatment was discontinued. Following this, his total sperm count gradually increased until it reached a total of 286 million in twenty-nine weeks (Fig. 1).

Case 2. D. A., aged 32, had a sterile marriage of two years' duration. During the last World War, he had malaria in the South Pacific. In 1943, a right herniorrhaphy was

[2] All spermatozoa counts herein reported refer to the total number in the ejaculate and not number per milliliter.

performed. There was nothing abnormal in the sexual history, nor in the physical examination. His height was 66 inches and weight 182 pounds. The testes, prostate, and prostatic fluid were normal. The results of routine laboratory tests and the basal metabolic rate were within normal limits. Several seminal fluid examinations revealed a moderate oligospermia of 45 million (Fig. 2). A testicular biopsy showed the basement membrane to be normal. There appeared to be an arrest of spermatogenesis at the spermatocyte level in about 50 per cent of the tubules. The Leydig cells appeared normal.

He was given 50 milligrams of testosterone propionate intramuscularly three times a

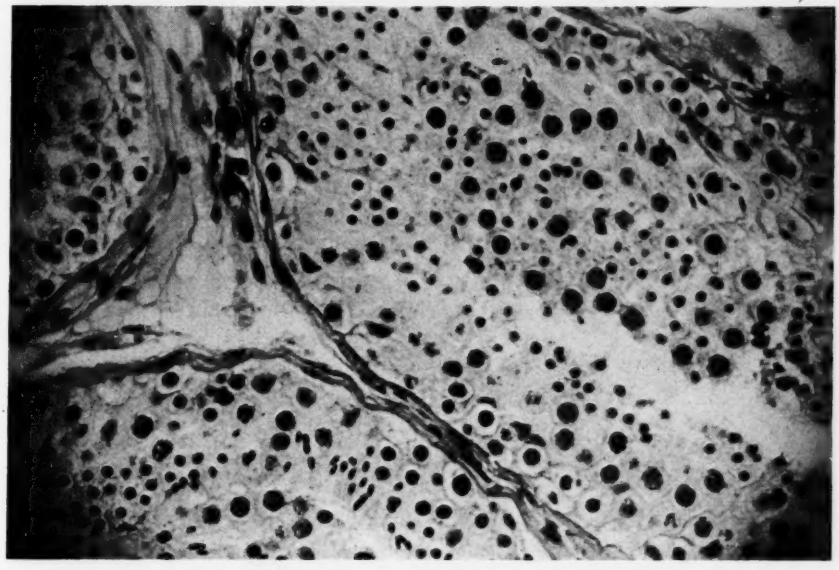

FIG. 4. *C. P., Case 3.* Histologic section of testicular biopsy specimen obtained before treatment (×420). Total sperm count 26 million. Focal areas of hypospermatogenesis with some thickening of basement membrane. Leydig cells appear normal.

week. After he had received 1,600 milligrams, he was nearly azoospermic and the treatment was discontinued. A gradual increase in the number of spermatozoa occurred until seventeen weeks later the sperm count had reached 103 million (Fig. 2).

Case 3. C. P., aged 32, had a barren marriage of three years' duration. The past history was noncontributory. There was nothing abnormal in the sexual history.

He was normally developed but overweight, with a height of 69 inches and weight of 205 pounds. The testes, prostate, and prostatic fluid were normal, as were the blood pressure, the blood count, and the results of urinalysis. Two basal metabolic rates were minus 13 and minus 18 per cent. Several seminal fluid analyses revealed a moderate oligospermia varying from 26 to 63 million (Fig. 3). He was given thyroid in a dose of 1 grain twice a day for two months without any improvement in the number or quality of the spermatozoa. A testicular biopsy specimen was obtained and a histologic section is shown in Figure 4.

Over a period of ten weeks, he received 1,500 milligrams of testosterone propionate.

At this time, he was practically azoospermic. Twenty-two weeks after discontinuation of the androgen, the total sperm count had reached 112 million. At this level, a second testicular biopsy was performed (Fig. 5).

Case 4. J. S., aged 27, had a barren marriage for six years. His family and past history, and the physical examination revealed no abnormalities. His weight was 178 pounds and height 71 inches. The testes, prostate, and prostatic fluid were normal. The routine labo-

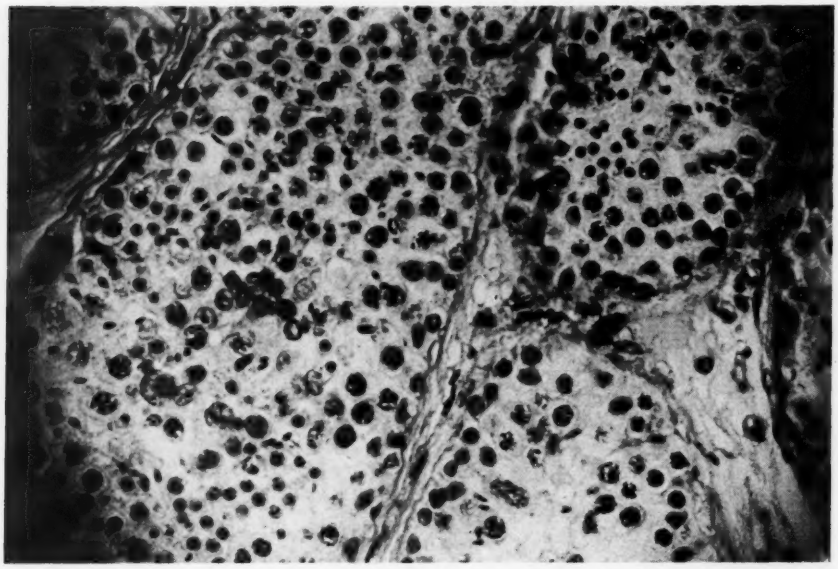

FIG. 5. *C. P., Case 3.* Histologic section of testicular biopsy specimen obtained twenty-two weeks after depression of spermatogenesis (×420). Total sperm count 113 million. Majority of tubules contain an appreciable number of mature spermatozoa. Spermatogenesis appears to be following normal pattern.

ratory tests, including two basal metabolic rates, gave results within normal limits. The seminal fluid analysis revealed a moderate oligospermia of 58 million (Fig. 6). A testicular biopsy was performed and the histologic pattern is shown in Figure 7.

He was given 2,550 milligrams of testosterone propionate during a 15-week period, after which his total sperm count had decreased to 1,520,000; testosterone propionate was discontinued. Sixteen weeks later the sperm count had risen to 118 million (Fig. 6). A second testicular biopsy was performed at this time. The results are shown in Figure 8.

Case 5. L. R., aged 31, had a sterile marriage of two years' duration. His family and past histories were irrelevant. The sexual history revealed that about 50 per cent of the time, he had no ejaculation during intercourse.

The physical examination showed nothing abnormal. His height was 71 inches and weight 162 pounds. The examination of the genitalia showed the right testis to be a little smaller than normal. The left testis was soft but about normal size. There was a mod-

erate sized left varicocele. The routine laboratory tests, including two basal metabolic rates, gave normal results. Seminal fluid analysis showed an oligospermia of 32 million (Fig. 9). A testicular biopsy was performed (Fig. 10).

He was given 2,500 milligrams of testosterone propionate in eighteen weeks, at the

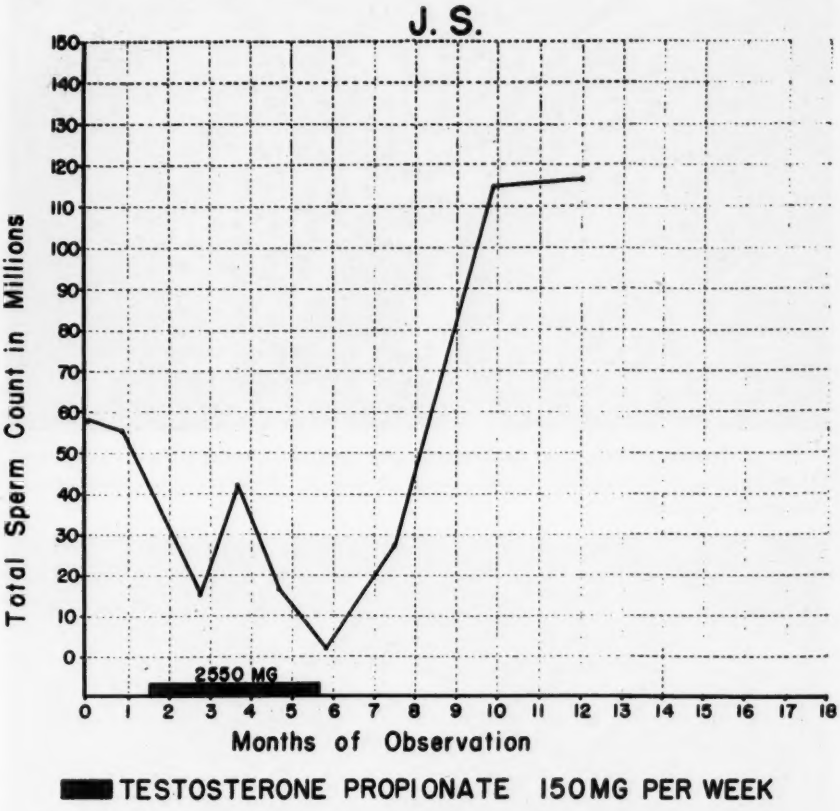

FIG. 6. *J. S., Case 4.* Showing rise of sperm count after termination of a course of 2,550 mg. of testosterone propionate.

end of which time he was azoospermic and treatment was stopped. Following this, there was a gradual increase in the number of spermatozoa until at the end of twenty-five weeks, the count had risen to 115,640,000 (Fig. 9). A second testicular biopsy was performed (Fig. 11).

DISCUSSION

In general, the 5 patients on whom this study was made were healthy, normal, well-nourished men. The testes, with the exception of Case 5, were normal grossly. No extrinsic cause could be uncovered to explain the

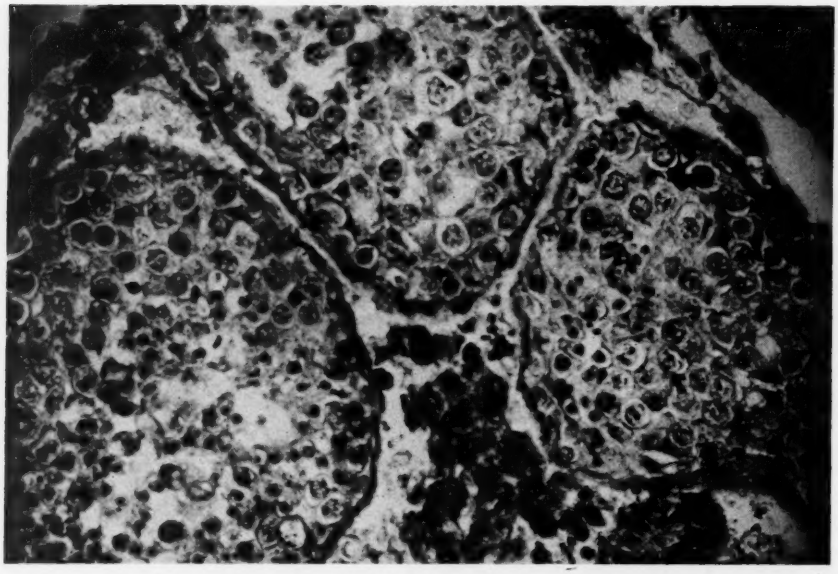

FIG. 7. Histologic section of testicular biopsy specimen obtained from *J. S., Case 4*, before treatment (×420). Total sperm count 58 million. Moderate hypogametogenesis. Potentially reversible.

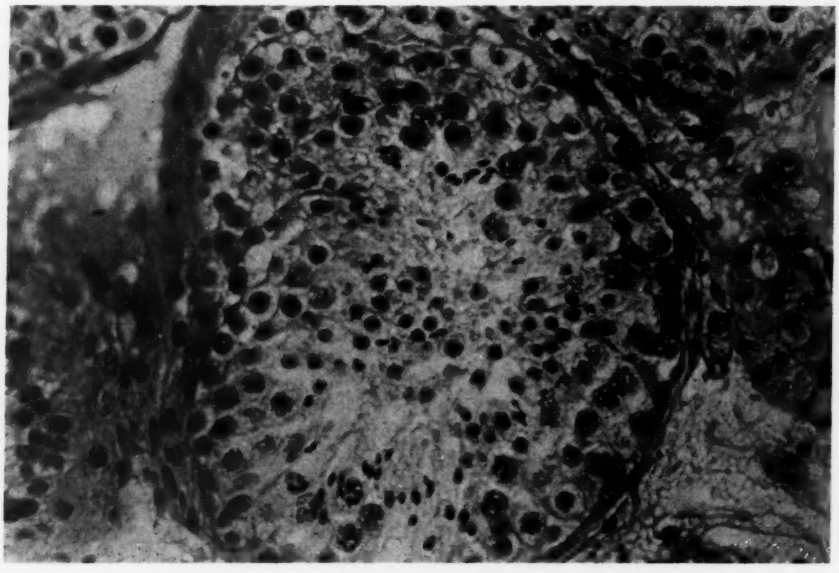

FIG. 8. Histologic section of testicular biopsy specimen obtained from *J. S., Case 4*, sixteen weeks after depression of spermatogenesis (×420). Total sperm count over 110 million. Note active spermatogenesis at all levels.

oligospermia. Initial biopsy specimens revealed spermatogenic arrest at various levels. The histopathologic changes in the tubules did not appear to be of an irreversible nature. During testosterone propionate therapy, spermatogenesis decreased in each patient until a stage of azoospermia or near-azoospermia was reached. Following discontinuance of treatment, in

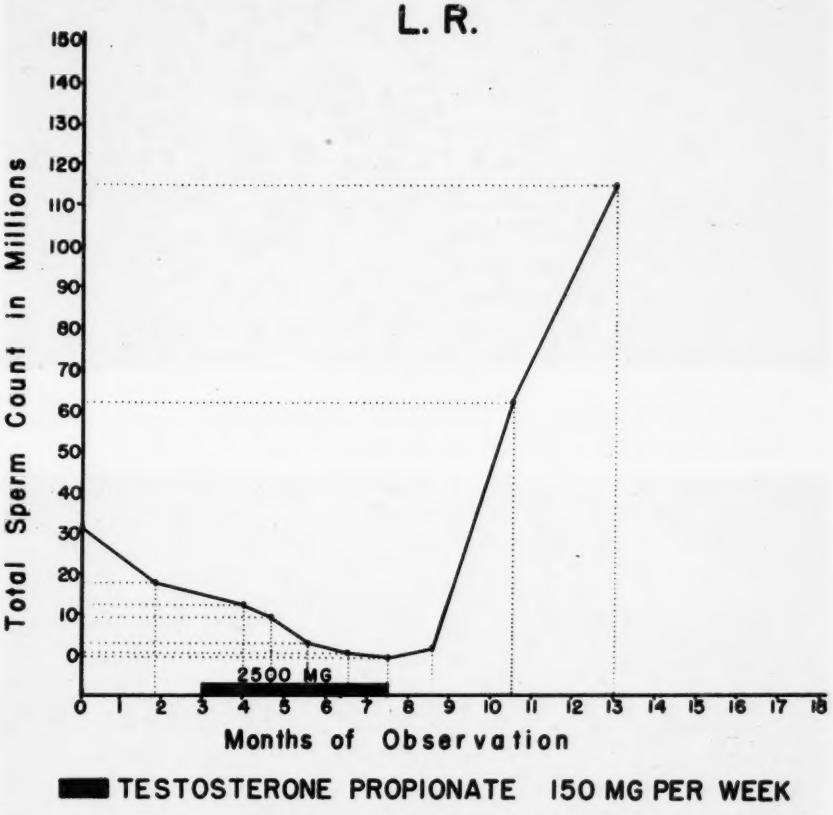

FIG. 9. *L. R., Case 5.* Azoospermia produced by 2,500 mg. of testosterone propionate, with subsequent rise in number of spermatozoa following cessation of treatment.

every instance there was an increase of spermatogenic activity, resulting in the production of mature spermatozoa in numbers far exceeding pre-treatment levels, In the 3 cases in which post-treatment biopsy specimens were obtained, the histologic picture showed an increase in the activity of the maturation processes as compared to the pre-treatment biopsy specimens.

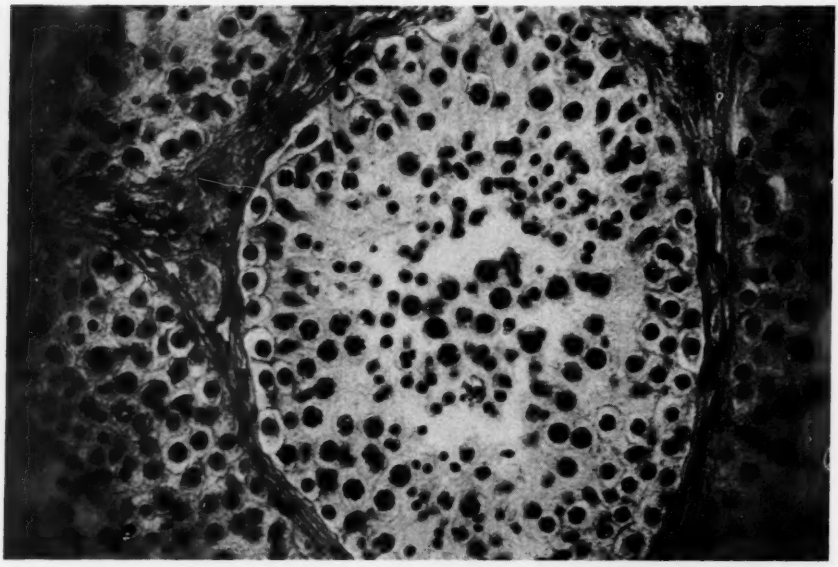

FIG. 10. Histologic section of testicular biopsy specimen from *L. R., Case 5*, before treatment (×420). Total sperm count 32 million. Spermatogenesis arrested at spermatocyte level in majority of tubules. Leydig cells appear normal.

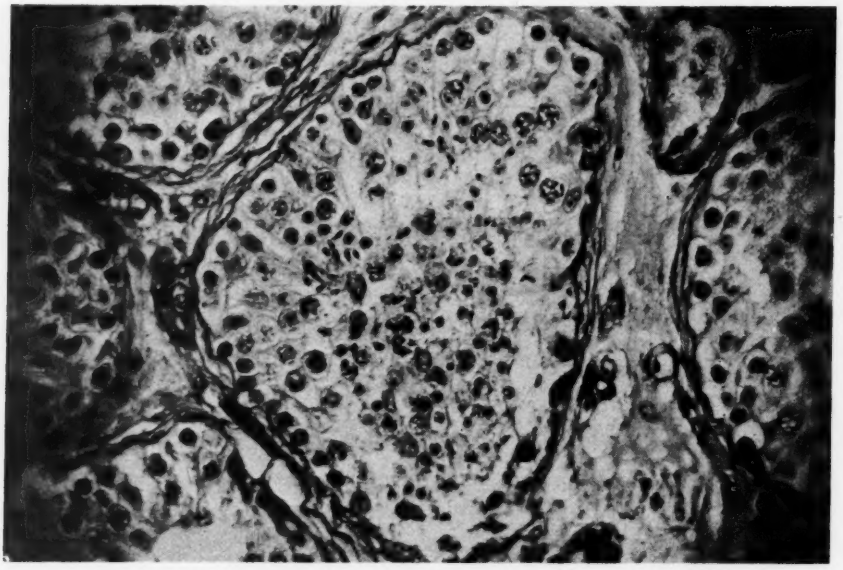

FIG. 11. Histologic section of testicular biopsy specimen from *L. R., Case 5*, taken twenty-five weeks after depression of spermatogenesis (×420). Total sperm count 115 million. Note active stages of sperm development.

Assays of urinary 17-ketosteroids and gonadotropins were not carried out in this study. Assays of this nature may contribute pertinent information which will clarify some of the mechanisms involved in the production of the phenomena observed; and studies of this kind are being undertaken in a similar group.

It is difficult at present for us to explain on a physiologic basis the improvement in spermatogenesis which occurred following the initial androgen-produced depression of the tubular function. At the present time, it is not known whether this response is peculiar to an androgenic substance, or whether further investigation will prove it to be a response which can be elicited by other agents.

SUMMARY

1. In 5 patients with oligospermia, spermatogenesis was further depressed to levels approaching azoospermia by the administration of testosterone propionate.

2. Following cessation of treatment, spermatogenic activity gradually increased until spermatozoa counts far exceeded pre-treatment levels.

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ISOLATION OF PREGNANEDIOL FROM HUMAN BILE AFTER ORAL ADMINISTRATION OF PROGESTERONE

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INDIRECT evidence that the liver is the site of conversion of progesterone to pregnanediol has been presented by numerous workers using a variety of methods. Selye (1) noted a heightened anesthetic effect of progesterone administered to partially hepatectomized rats, compared to its effect in intact rats. Dosne (2) showed that pellets of progesterone implanted into the spleen of an estrogen-treated guinea pig were less antifibromatogenic than pellets of progesterone implanted subcutaneously and concluded that the liver was the site of inactivation of progesterone. Kochakian, Hoskins, and Bruce (3) found in female rabbits that progesterone pellets implanted in the mesentery were less effective in producing an endometrial response than pellets implanted subcutaneously or in the muscle. Masson and Hoffman (4) found in rabbits that it required 200 mg. of progesterone by gavage to obtain a progestational effect comparable to that elicited by 0.25 to 0.5 mg. administered subcutaneously. In the partially hepatectomized rabbit, however, comparable progestational changes were obtained by the administration of 25 mg. of progesterone by gavage.

Few studies have been made on bile in an attempt to show by more direct means the role of the liver in the conversion of progesterone to pregnanediol. Pearlman and Pincus (5) administered massive oral doses of Δ^5-pregnenol-3(β)one-20 to a postmenopausal woman and recovered minute amounts of pregnanediol-3(α), 20(α) from the bile. Pearlman and Cerceo (6) isolated pregnanol-3(α)one-20 and pregnanediol-3(α) 20(β) from the gallbladder bile of pregnant cows.

It is the purpose of this paper to report the isolation of pregnanediol from human bile after the oral administration of progesterone and thus to offer direct evidence of the role of the liver in the metabolism of progesterone.

EXPERIMENTAL

Four patients (3 males and 1 female) who were operated upon for relief of biliary obstruction, served as subjects. On the third or fourth day after establishment of T-tube biliary drainage, each subject was given 500 mg.

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of crystalline progesterone orally.[1] In 1 patient the material was given as an aqueous suspension; in the others, the powdered progesterone was contained in a capsule. Twenty-four-hour collections of bile and urine were obtained for five days and stored in a refrigerator. In the initial experiments, the bile was extracted with ether and both the ether extract and the residual bile were analyzed for pregnanediol. It soon became apparent that the larger quantities of pregnanediol were being obtained from the ether-insoluble fraction. The following procedure based on the method of Astwood, Grady, and Wermer (7) was evolved:

To the entire bile specimen were added 2 volumes of toluene and 1/10 volume of 18 N sulfuric acid and the mixture was boiled under reflux for fifteen minutes. After cooling and shaking, the aqueous layer was separated and reboiled for fifteen minutes with fresh toluene. The two extracts were then treated separately. They were washed twice with 2 N sodium hydroxide and two to four times with hot distilled water. The extracts were poured into Erlenmeyer flasks and excess water boiled off on a hot plate. A column was made, using aluminum oxide ($6\frac{1}{2}$ grams of water/100 grams) about 4 cm. deep in a 2-cm. diameter sintered glass funnel. Twenty cc. of toluene was added, the alumina was suspended by vigorous mixing with a spatula and then allowed to settle. The aluminum oxide was covered with a layer of sand, care being taken not to disturb the column. The toluene extract was poured into the funnel and drawn through by gentle suction. After the extract had run through, the column was washed with 10 cc. of toluene, and then with 0.5 per cent absolute alcohol in toluene. These fractions were discarded. The desired fraction was then eluted with 3 per cent absolute alcohol in toluene, at least 100 cc. being used. The extract was evaporated to dryness and washed three times with petroleum ether. The petroleum ether fraction was centrifuged. Any remaining precipitate in the centrifuge tube and the residue from the washing with petroleum ether were dissolved in hot 95 per cent alcohol, and evaporated to a volume of 3 to 5 cc. Three volumes of water were added and the solution boiled gently. It was then allowed to stand at room temperature for twenty-four hours to obtain maximum precipitation. If the precipitate was not white, it was redissolved in hot 95 per cent alcohol and reprecipitated and refiltered. When colorless, the precipitate was dissolved in alcohol and transferred to a small vial. The crystals obtained were sent to Dr. Konrad Dobriner, who kindly carried out infra-red spectroscopy.

RESULTS

In each of the 4 patients with T-tube biliary drainage, small amounts of a white crystalline substance were obtained from the bile within the first twenty-four hours after the oral administration of 500 mg. of crystalline progesterone. In all but one instance the amounts recovered were insufficient for accurate weighing. The largest amount recovered was 3.8 mg. Analysis of the bile obtained on the second day after oral progesterone disclosed crystals on only one occasion. Each of the specimens obtained was shown by infra-red spectroscopy to contain pregnanediol. The speci-

[1] Generously supplied by the Schering Corporation, Bloomfield, New Jersey.

men weighing 3.8 mg. was reported as containing pregnanediol but it was not pure. The sensitivity of the method was evaluated by adding known amounts of sodium pregnanediol glucuronidate to samples of bile. The results are shown in Table 1.

TABLE 1

Amount of sodium pregnanediol glucuronidate added, mg.	Volume of bile, cc.	Theoretical yield, mg.	Actual yield, mg.	Percentage recovery
100	100	44.7	19.4	43
100	300	44.7	29.1	65
100	330	44.7	6.6	15
100	220	44.7	16.0	36

Attempts were made to demonstrate progesterone in the bile specimens from which pregnanediol was isolated. Adult female rabbits were castrated and given 5 micrograms of stilbestrol daily for five days. Beginning on the sixth day, 1 rabbit received 1 cc. of bile intramuscularly daily for five days. On the eleventh day the abdomen was opened and a 1-cm. segment of the uterus was removed, fixed and stained with hematoxylin and eosin. No progestational effect was observed. The same rabbit similarly prepared with stilbestrol failed to show progestational response in the uterus after 2 cc. of bile daily for five days. In a third experiment, the entire 24-hour bile specimen was extracted three times with ether and the extract evaporated to dryness. The residue was dissolved in 15 cc. of peanut oil and the entire amount was injected over a 5-day period into an estrogen-primed rabbit. No progestational effect was observed. The estrogen-primed uterus of the same rabbit subsequently showed a full progestational response to 1 mg. of crystalline progesterone over a period of five days.

DISCUSSION

The isolation of pregnanediol from human bile after the oral administration of large doses of progesterone is offered as direct evidence that progesterone is inactivated or converted to pregnanediol in the liver. It has been known for some time that bile contains estrogenic material and Cantarow *et al.* (8) have demonstrated that large quantities of estrogen are excreted in the bile but that none can be found in the liver, spleen, intestinal wall and hepatic vein blood. These findings have led Cantarow and his coworkers to suggest an enterohepatic circulation of the estrogens.

The isolation of pregnanediol from bile implicates the liver as the site of conversion of progesterone to pregnanediol, but the failure to demonstrate progestational activity in the same bile indicates that conversion must be complete and fairly rapid and that an enterohepatic circulation does not occur in the case of progesterone. Indirect evidence of pregnanediol excretion in bile was obtained by the observation that in 2 patients given progesterone while the common duct was obstructed, the urinary excretion of pregnanediol was significantly greater. This phenomenon, also observed by Hoffman and coworkers in the rabbit (9), indicates an altered route of excretion in the presence of common duct obstruction.

The results obtained indicate that the method of pregnanediol determination of Astwood, Grady, and Wermer can be adapted to analysis of bile. It was found that the usual method of hydrolysis by boiling with 100 cc. of toluene and H_2SO_4 did not extract all the pregnanediol. The use of twice the amount of toluene and repeating the extraction resulted in larger yields. The variable percentage recovery in the experiments in which known amounts of pregnanediol were added to bile, remains unexplained. In one experiment, 100 mg. of free pregnanediol were added to bile and 45 mg. were recovered, suggesting that much of the loss entailed is in the process of extraction. Bile, of course, contains many steroids, cholesterol being the most abundant. The differential solubility of cholesterol and pregnanediol in petroleum ether was satisfactorily utilized to separate out pregnanediol.

SUMMARY

Following the oral administration of progesterone, small amounts of pregnanediol were isolated from the bile of human subjects in whom T-tube biliary drainage was established for the relief of common duct obstruction. This finding implicates the liver in the conversion of progesterone to pregnanediol. The inability to demonstrate progestational activity in the bile from which pregnanediol was isolated suggests that progesterone does not undergo an enterohepatic circulation as has been described for estrogens.

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PREGNANEDIOL DETERMINATIONS IN THE CLINIC AND IN RESEARCH*

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PREGNANEDIOL was first isolated by Marrian (1) in 1929, when he was engaged in the purification of "oestrin" from pregnancy urine. He described the properties of this new "unidentified solid alcohol" very precisely, and his first analyses came surprisingly near to the right formula, $C_{21}H_{36}O_2$, at a time when the greater part of steroid chemistry was still unknown. To-day, looking back on twenty years of chemical, biologic, and clinical research, it is comparatively easy to see the principal features of the interesting pattern which centers around the pregnanediol molecule.

The first clue to the biologic significance of pregnanediol is given by its structural formula which shows a striking resemblance to that of the corpus luteum hormone, progesterone. Pregnanediol is nothing else but reduced progesterone, with all the double bonds of the latter saturated by hydrogen. Thus, the obvious inference would be that *urinary pregnanediol is a metabolite of progesterone.* This important fact is the reason why so many workers are interested in pregnanediol determinations.

But *is* it a fact? The evidence is fully conclusive that it is, as the following brief summary shows:

1. In men (2, 3) and in normal menopausal women (3, 4), urinary pregnanediol is found only after injection of progesterone.

2. In normal young women, urinary pregnanediol is found only when the progesterone-producing organs, placenta or corpus luteum, are present.

It is only fair to state that these fundamental facts must be qualified in two respects. First, pregnanediol is not the *only* metabolite of progesterone. Although, to my knowledge, no other substance except pregnanolone-3α, 20 has ever been isolated in metabolism studies (5), it has often been assumed that the pregnanediol stereoisomers and the pregnanolones of pregnancy urine are derived from progesterone—to say nothing of the fact that the pregnanediol excreted after progesterone injection accounts for only about 10 to 15 per cent of the injected dose.

Moreover, not only progesterone but also desoxycorticosterone (6), cholesterol (7), and a pregnenolone (8) have been shown to undergo metabolic transformation into pregnanediol in the human organism. Al-

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though these findings are pertinent, they must not be allowed to confuse the main issue. According to our present knowledge, only the adrenal cortex could be responsible for the elaboration of *all* of these pregnanediol precursors. As a matter of fact, adrenal hyperfunction *is* sometimes associated with increased urinary pregnanediol values (9). But the fundamental fact that pregnanediol reflects the presence of progesterone, still holds good since, in normal individuals, the amount of pregnanediol due to adrenal function is too small to interfere with pregnanediol determinations by ordinary methods in twenty-four hour collections of urine.

METHOD

Everybody will readily agree with the general principle that, in the clinic as well as in research, there should be some certainty regarding the substance or substances which are estimated by the method employed. In other words, the specificity of methods is of primary importance. Just as the only real proof of cancer depends upon the microscopic detection of malignant cells, the presence and amount of a chemical compound has to be determined by its isolation and chemical identification. In clinical routine, this can rarely be done. Direct methods are superior to indirect estimations, which are more liable to errors of overestimation and contamination. Pregnanediol is excreted in a conjugated form, as the sodium salt of its glucuronide. The numerous procedures described for its estimation can be divided into three major groups, according to whether a glucuronidate fraction, glucuronic acid, or a pregnanediol fraction is used for the decisive reading. Speaking for myself and my co-workers, the third group is definitely preferable, because it is direct.

Since most of the results to be presented have been obtained by our own method (4, 10), a short survey of the procedure and its reliability follows. After acid hydrolysis of the urine, a neutral steroid fraction in toluene solution is obtained by the well known Astwood-Jones technique. The polyhydroxy compounds of this extract, including pregnanediol, are adsorbed on a chromatographic column containing aluminum oxide, and the pregnanediol fraction is then eluted with a benzene-alcohol mixture. Upon washing the elution residue with cold ether to remove the greater part of the pigments, the pregnanediol crystallizes, can be weighed, and, if desired, identified by chemical methods (Fig. 1). The technique is rather simple and requires nothing more than an analytic scale and ordinary glassware. Any laboratory assistant can do it, if supervised by a chemist with a knowledge of chromatography. One person can easily make an average of 20 determinations weekly.

The reliability as well as the limitations of the method are demonstrated by the following four points. 1. The substance isolated in the routine

procedure has been identified chemically as pregnanediol-3α, 20α. 2. Recovery of crystalline pregnanediol added to urine is practically complete, whereas a loss of 30–35 per cent may occur through hydrolysis of the glucuronide complex. 3. Duplicates done by four different operators vary, on the average, by ± 7.2 per cent from their mean. Four fifths of these deviations are less than 15 per cent. 4. The lower limit of the method is at about one half of a milligram.

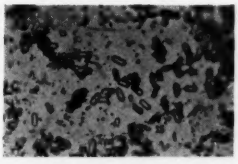

FIG. 1. Photomicrograph of pregnanediol fraction obtained in a routine determination by the chromatographic method.

COMMENT

At the British Congress of Obstetrics and Gynecology in London in July 1949, there was much scepticism concerning the practical significance and usefulness of pregnanediol determinations in obstetrics. It cannot be denied that the reports of different authors on this subject are partly contradictory. However, according to our experience, the greater part of those discrepancies might perhaps be attributed to three major causes. The first is the question of methods, a question which cannot be overemphasized. We have already discussed it, and we shall have opportunity to refer to it again in a practical illustration. One important part of it is the absolute necessity to work with complete twenty-four-hour collections of urine, not with morning or night specimens. The second cause for contradictory findings may arise from a tendency to generalize. There is, for instance, a certain variety in the etiology of abortion, and it is not justified to assume *a priori* that all kinds of abortion must be related to abnormal hormone levels. The third cause is, to a certain extent, similar to the second: it is the question of applicability. Decisive information from a laboratory test cannot be expected if the level of the substance being determined reflects only slightly or not at all the biologic event you want to detect.

Normal pregnancy

As an illustration, let us consider the pregnanediol values of normal pregnancy. The results of different workers (11, 12) agree at least in that

the values during the first months are of the same order as those found during the menstrual cycle. For this reason, a pregnanediol determination can not be a truly reliable test for early diagnosis of pregnancy. The claim that it is (13, 14) has been put forward, it is true, with the additional caution that the test works only in cases of amenorrhea after very regular normal cycles. We could agree, if normal regular cycles were more common, but unfortunately we can never determine with certainty the moment of ovulation or the duration of the luteal phase. Therefore, it is preferable to base early diagnosis of pregnancy upon the detection of a substance like the gonadotropins, which are low in menstrual cycles, but decisively high after implantation of the ovum.

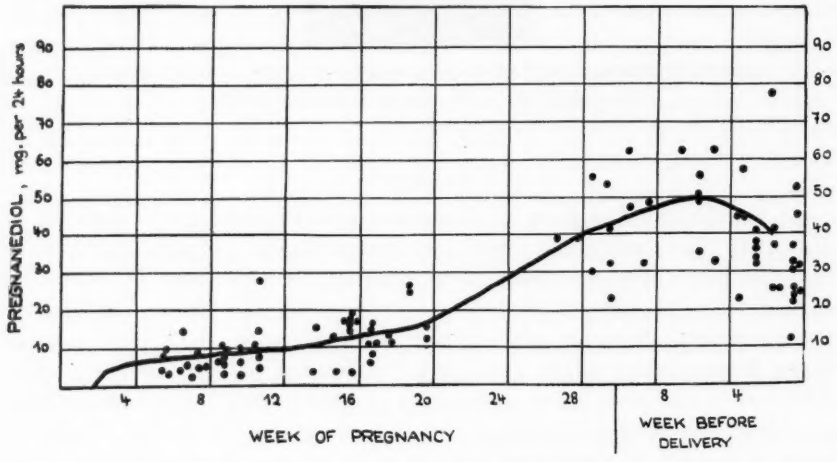

FIG. 2. Pregnanediol excretion in normal pregnancy. Scatter diagram of 87 determinations in 52 cases. The values of the last three months are plotted backwards from the date of delivery. The curve connects the monthly means.

Our pregnancy values after the fifth month until term are appreciably lower than those of some other workers, but they are in good agreement with Marrian's results (15). These discrepancies of absolute values can be explained, at least partly, by the varying degree of specificity in different methods. The mean curve flattens abruptly at the eighth month. This is due to the fact that, even in normal pregnancies, there is a certain variation in the dates of delivery as estimated from the date of the last menstruation. The well known peak in pregnanediol excretion, several weeks before delivery, emerges immediately on plotting our values of the last three months backwards from the date of birth (Fig. 2). This latter curve, therefore, seems to represent the true mean values of normal full-term pregnancies, as obtained by the chromatographic method.

Abortion

Several disturbances of pregnancy are associated with pregnanediol levels lower than normal. In threatened abortion and threatened premature labor with favorable outcome, most of the values are below our average

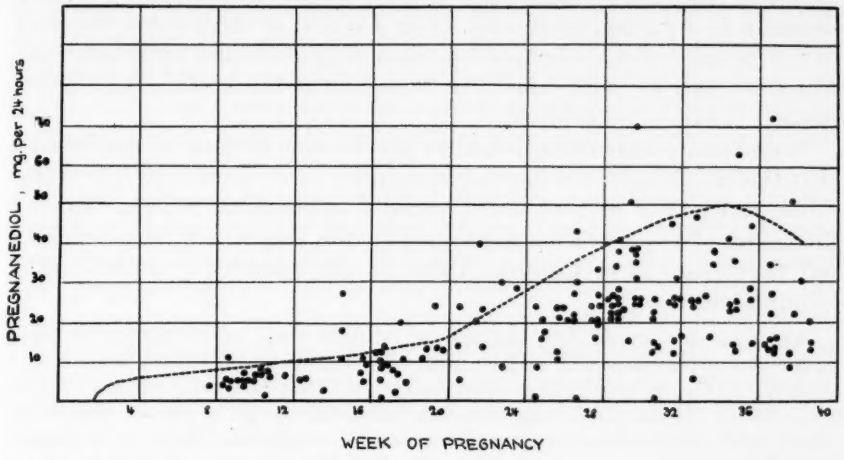

FIG. 3. Pregnanediol excretion in threatened abortion and threatened premature labor with favorable outcome. The dotted line is the mean curve shown in Figure 2.

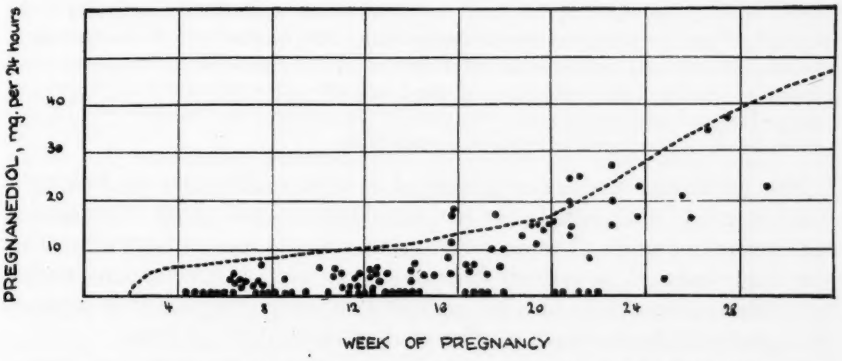

FIG. 4. Pregnanediol excretion in spontaneous abortion. The dotted line is the mean curve shown in Figure 2.

curve, but on the whole not very much lower than the inferior limit of the latter's variability (Fig. 3). The same tendency is more striking in spontaneous abortion, in which case urinary pregnanediol is frequently extremely low or even absent (Fig. 4).

Two important qualifications must be made with respect to our results.

First, the greater part of our values refer to cases in which the physical findings were such that the outcome of the pregnancy was doubtful. For instance, we did *not* determine pregnanediol in all the cases in which abortion was certain. It is possible, therefore, that our material does not do full justice to the *biologic* relationship between pregnanediol and progesterone secretion in disturbed pregnancy. Over a period of three years, we tried to use pregnanediol determinations as a diagnostic and prognostic aid. Therefore, our material reflects to some extent the applicability of the method in every-day practice.

The second consideration is a more general one. It must be emphasized that this whole subject is much too complex to be condensed into a few tables and graphs without losing valuable information. A more detailed discussion of the problems mentioned in this report will be possible in our papers now in preparation. However, the following is a particularly informative case:

A woman, 40 years old, had been bleeding for three weeks before entering the clinic at the end of the eighteenth week of pregnancy. The Aschheim-Zondek reaction was positive, and the first pregnanediol value was normal. The second value, obtained during progesterone and vitamin E treatment, was rather high—possibly due to a vitamin E effect which will be discussed later. She seemed to respond favorably to treatment; hemorrhage ceased three days after admission, and nine days later she left the clinic, with pregnancy apparently developing normally. On the day of discharge, Aschheim-Zondek and pregnanediol tests were repeated. The response to the former was positive again, but pregnanediol excretion had suddenly dropped well below 50 per cent of the normal mean. After receiving this result, we wrote to this woman that we had reason to suspect her pregnancy was not developing normally. As a matter of fact, she had begun bleeding intermittently immediately after she had left the clinic. About two weeks later, premature rupture of the membranes occurred, and after a few days she was delivered of a premature underdeveloped fetus. One third of the placenta was covered by old blood clots with compressed anemic cotyledons underneath.

This particular case has been selected in order to illustrate the following conclusions. In threatened abortion, a sudden or steady drop of pregnanediol values in a series of repeated determinations is more informative than one single value. This sudden drop may occur while the Aschheim-Zondek reaction is still positive. Low pregnanediol values are frequently associated with placental damage and insufficient development of the fetus.

There is no rational purpose in giving progesterone treatment to patients whose pregnanediol excretion is normal. In addition, since the customary intramuscular doses of progesterone, up to 20 mg. daily, fail to raise appreciably the low pregnanediol values in threatened abortion, there is no justification for assuming that our doses are sufficiently high to bring the hormone level up to the normal range as reflected by pregnanediol excretion. Some consideration may be given to the idea that progesterone of placental origin acts, so to speak, locally, whereas injected progester-

one is distributed in the circulation before reaching the uterus. Moreover, the possibility must not be overlooked that injected progesterone may not be metabolized in the same way as the endogenous hormone.

As previously stated, extragenital causes of abortion may not necessarily be reflected in decreasing hormone levels. Thus, in our cases of abortion accompanied by fever or caused by mechanical manipulations, the pregnanediol values are not consistently low, but vary around the mean curve; they drop to zero, of course, when the ovum is already dead or detached.

The stilbestrol treatment of threatened or repeated abortion was introduced by the Smiths (11) with spectacular and probably unexpected clinical success. In one of our cases, a woman received 25 mg. of stilbestrol daily and pregnanediol disappeared completely from the urine, but appeared again immediately when the treatment was discontinued. At that time we were puzzled by this observation, since the Smiths had claimed that pregnanediol excretion *increases* under stilbestrol treatment. The answer to our riddle seemed to be given by the work of Davis and Fugo (16) and of Sommerville and co-workers (17), suggesting that the rise of urinary pregnanediol, as detected by the Venning method, is *simulated* by stilbestrol itself. Pregnanediol, on the other hand, *decreases* under stilbestrol treatment, exactly as we had observed. This practical illustration stresses the importance of specific methods.

Pregnanediol determination compared with biologic tests, in pregnancy and threatened abortion. In 123 cases, we made a pregnanediol determination simultaneously with an Aschheim-Zondek test in 58 cases, with a Friedman test in 52 cases, and with a Hogben test in 13 cases. For the sake of this comparison, a pregnanediol value is labeled "positive" if more than, and "negative" if less than, 50 per cent of the corresponding value on our mean curve. We call results "correct" if they are positive in normal pregnancy and threatened abortion with favorable outcome, and if they are negative in abortion cases. The correspondingly opposite results are termed "incorrect." Using these definitions, pregnanediol and biologic tests were both correct in 50 cases, and both incorrect in 35 cases. In the remaining 38 cases the biologic tests were incorrect 28 times, whereas the pregnanediol test was incorrect only 10 times. Of course it is not surprising to find that the chemical test, being quantitative, had a greater chance of prognostic success than the biologic pregnancy tests, which can be used only qualitatively in our clinical routine. These 123 cases, however, illustrate another fact of far greater importance. They show that we must not jump to conclusions on the basis of laboratory results alone. Most of these results acquire their real value only when set into the right perspective, that is, when considered in close relation to the whole clinical picture of a case.

Ectopic pregnancy and hydatid mole

We may dispense briefly with ectopic pregnancy and hydatid mole. In ectopic pregnancy we found most values to be rather low or zero, either because living conditions for the chorionic villi were unfavorable, or because the ovum had already become devitalized at the time of determination. It may be worth mentioning that in 3 of our few cases of hydatid mole, pregnanediol was present, and was even abnormally high in 1 case.

Toxemia

A disorder of pregnancy which remains the most mysterious and fascinating of them all is toxemia. The reports (18, 19, 20) on pregnanediol ex-

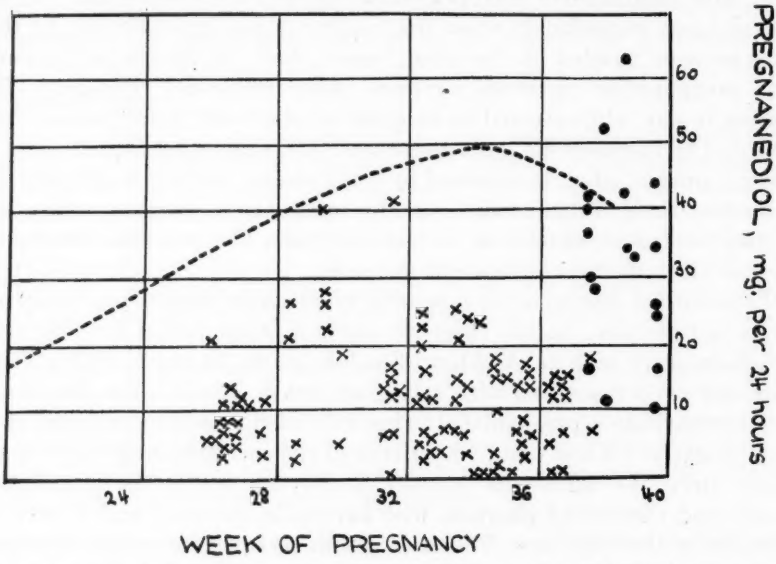

FIG. 5. Pregnanediol excretion in toxemia. • Child normal; x Child dead or underdeveloped. The dotted line is the mean curve shown in Figure 2.

cretion in toxemia are somewhat contradictory and, judging from our results, it seems understandable that they should be. Obviously, there are many low values, but also a number of normal ones, and no general trend seems to be present. Not satisfied with that, we separated our material into two groups. The first group contains the toxemia cases in which a normal child was born, and covers most of the normal range values. In the other group, we assembled the cases in which the child was born dead or underdeveloped. Using this criterion, the separation of normal from low pregnanediol levels becomes surprisingly clear (Fig. 5).

In 1 representative case of the second group, all the typical features were present—urinary albumen, high blood pressure, and pregnanediol excretion well below normal (Fig. 6). The patient did not respond to treat-

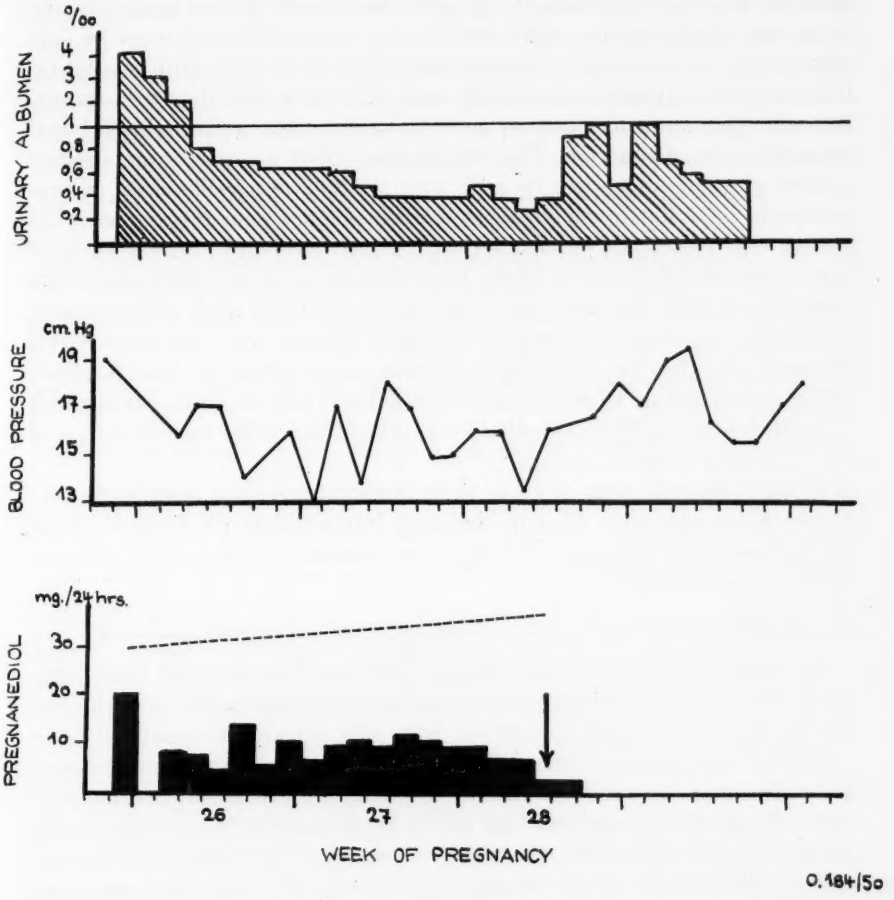

FIG. 6. Daily values of urinary albumen, arterial blood pressure, and urinary pregnanediol, in a case of toxemia of pregnancy. The dotted line is the mean curve shown in Figure 2. The arrow indicates the date of interruption.

ment, and interruption of pregnancy became necessary in the twenty-eighth week. The child was born dead and weighed 800 grams. The placenta showed a hematoma as well as white and red infarcts. It is interesting to note that there is no strict correlation between pregnanediol excretion and blood pressure or urinary albumen. We have several cases in which this lack of correlation is even more obvious.

There are three plausible explanations for low pregnanediol excretion in severe toxemia: namely, that the progesterone production may be low; that the metabolism of this hormone may be altered; or that the excretory function may be disturbed. At present, we simply do not know enough to be sure which one, or which combination, of these factors must be held responsible. At first sight it seemed attractive to try the third possibility, linking the low pregnanediol levels with the renal disorders of toxemia. Actually, pregnanediol does not seem to be directly correlated with blood pressure or renal function. This was confirmed by a series of metabolism studies of which more will be said later. In 30 comparable cases of progesterone injection in menopausal women, a very poor pregnanediol recovery was frequently associated with renal deficiency; but in spite of marked renal deficiency or slight liver damage, good recovery was sometimes found. One case of a patient in the thirty-third week of pregnancy, suffering from hydronephrosis of the right kidney, was very instructive. Immediately after the usual 24-hour collection of urine, we took a 2-hour catheterized sample from the nephrotic kidney, and much to our surprise it yielded a normal pregnanediol excretion, which even exceeded that in the preceding 24-hour sample.

From an overall point of view, these observations seem to indicate that placental insufficiency, that is, deficient progesterone production, is the chief cause of low pregnanediol values in toxemia as well as in other pregnancy complications.

Menstrual cycle

As suggested by its name, pregnanediol was first isolated from pregnancy urine, and therefore most reports on this compound deal with pregnancy. No less interesting, however, is its rôle during the menstrual cycle.

We followed daily pregnanediol excretion during 3 cycles in the same normal woman. In accordance with corpus luteum function, pregnanediol is not found during the first half of the cycle and the level drops to zero at, or immediately before, the onset of bleeding. The daily variations are great, low levels are rather frequent, and even a zero value may occur occasionally. Thus, the *amount* of pregnanediol found in one single determination would not reflect the degree of corpus luteum activity. The total amount excreted during one entire cycle might perhaps provide such quantitative information, but practically, patients willing and able to cooperate in the task of obtaining complete two-week urine collections cannot be found in large numbers. In gynecologic practice, we have to be satisfied with the qualitative information obtained from one or several determinations.

We have assembled the results of about 140 determinations in 100 cycles.

They were normal cycles, ranging from three to five weeks, as far as the menstrual histories were concerned. Although this heterogeneous material shows an even greater variability than the 3 cycles of the same woman, no pregnanediol was detected before the thirteenth day. Of the values obtained, the higher ones were of the same order as early pregnancy levels. Although great variability was to be expected, the numerous zero values during the second half of the cycle claim special attention. They are a consequence of two different factors. One of them is the variation in cycle lengths. This factor is eliminated when the points are plotted backwards

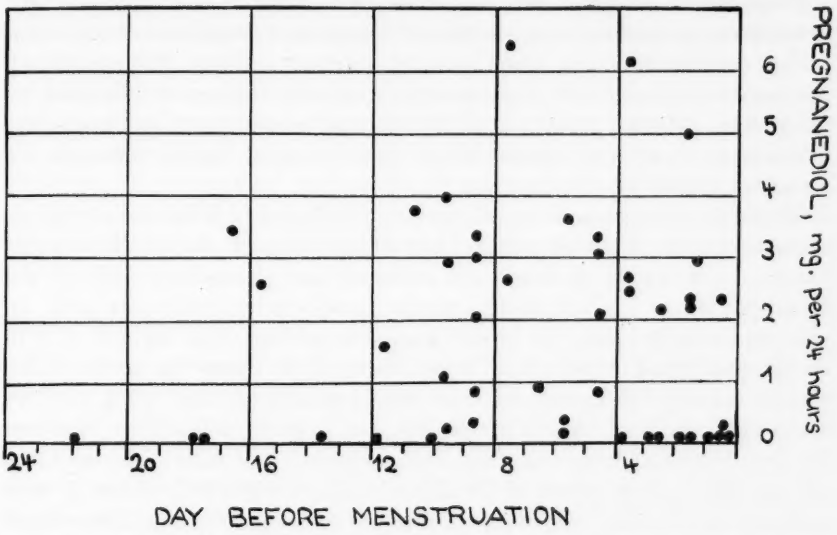

FIG. 7. Pregnanediol excretion during the menstrual cycle. Scatter diagram plotted backwards from the day of the next menstruation.

from the first day of the next bleeding, whereupon some of the zeros fall on the days preceding menstruation (Fig. 7). The other factor concerns zero values indifferent to graphic manipulations. They are evidence that apparently normal menstruations may well be associated with luteal deficiency or anovulatory cycles perhaps more frequently than is generally realized. In these cases, just as during the normal proliferative phase, no urinary pregnanediol is detected by the chromatographic method.

We experienced a practical lesson in this phase of endocrinology when 2 healthy laboratory assistants, who had offered themselves for study during a complete cycle, gave zero results in some 60 determinations.

Uterine myoma. The pregnanediol values during the cycle seem to indicate a difference between myomatous and the normal cases. In animals,

hyperproliferation due to estrogens can be inhibited by progesterone treatment (21). We thought, therefore, that myoma might be associated with luteal deficiency. We want to accumulate a little more material before trying a thorough analysis of the cases, including statistical treatment, but already it seems that all of the myomatous cases show lower pregnanediol levels than several of the normal ones. If this result be confirmed, it will be in good agreement with our histologic findings (22) indicating progestational deficiency in the endometrium of many myomatous patients.

Pregnanediol determination compared with endometrial biopsy findings. A comparison between the histologic picture and pregnanediol excretion has its own merits quite apart from the myoma problem. By comparing the results obtained with our chemical tool with the results obtained by the well-established procedure of endometrial biopsy, we may obtain real evidence as to whether pregnanediol determinations during the cycle *are* a test for corpus luteum function.

When classifying our material, we found 64 cases in which biopsy specimens had been obtained within forty-eight hours of urine collection for pregnanediol. In 26 of these, the endometrium showed no signs of the luteal phase, the mucosa being atrophic, proliferative, or hyperplastic. In the remaining 38 cases, the luteal phase was evident, just beginning, or of the premenstrual or menstrual type. Forty-seven times the pregnanediol results were in full agreement with the histologic picture, being positive when progestational signs were present, and negative when they were not. In the discordant cases, we found pregnanediol in 1 case of endometrial atrophy when no response of the tissue could be expected; and in 2 cases without endometrial response, despite the presence of an active corpus luteum as proved by ovarian biopsy. No pregnanediol was found in 7 cases with (pre-)menstrual mucosa, a finding which is in good agreement with the corresponding pregnanediol decrease already discussed. Finally, no pregnanediol was found in 5 cases in which the endometrium showed only the very first signs of beginning luteinization; it seems that the small amounts present at that time are not always detected by the chromatographic method. This leaves us with 2 unexplained results in which no urinary pregnanediol was present in spite of full luteinization. They are a warning that *one negative* result, even around the twentieth day, is not conclusive. In order to reduce the risk of such false negatives, it is advisable to repeat the determination and to use 48-hour collections.

We were able to confirm Brown and Bradbury's interesting findings (23) in 4 cases (24). In normal cycles, bleeding is suppressed or delayed and the symptoms of luteal activity are maintained or accentuated, if the corpus luteum is preserved and stimulated by continuous huge doses of

chorionic gonadotropin. Supported by histologic control, this experimental series demonstrated very nicely the parallelism between the state of the corpus luteum, endometrial response, and pregnanediol excretion.

All this seems to leave no doubt that pregnanediol determination is indeed a reliable test for corpus luteum function. We have used it, for instance, in many cases of abnormal uterine bleeding. Such patients reveal very frequently complete absence of pregnanediol which, consequently, would seem to reflect luteal deficiency. On the other hand, the pregnanediol test has been used to confirm the diagnosis of persistent corpus luteum (25).

In marital sterility the pregnanediol test seems to give more specific information with respect to luteal activity, than the vaginal smear. The chemical test is complementary to premenstrual endometrial biopsy and may even replace it in cases in which it is desirable to avoid the latter's inconvenience. The objection might be raised that a slight progesterone secretion may be present just before, or even without ovulation, and this possibility cannot be excluded. If, however, we do find a definitely high pregnanediol level, then follicle rupture and corpus luteum formation are practically certain.

Clinical application

Urinary pregnanediol is the last link of an intricate physiologic chain reaction (26) involving, in this order: production of follicle-stimulating hormone (FSH); follicle growth; production of interstitial-cell stimulating hormone (ICSH); estrogen production; ovulation; corpus luteum formation; prolactin production, and finally, functional corpus luteum with progesterone production, which, in turn, is reflected by the metabolite, urinary pregnanediol. This final effect depends entirely upon the processes preceding it, and it can emerge only if all the other steps are intact. Corpus luteum activity is at the top of this functional hierarchy. The detection of pregnanediol gives implicit information that ovarian as well as pituitary gonadotropic functions are in order.

From a purely clinical standpoint, most of what we have discussed until now, boils down to the following statement (27): pregnanediol levels are increased in normal pregnancy, in cases of persistent corpus luteum, and in theca-cell and certain adrenal tumors; little or no pregnanediol is found in missed or threatened abortion, in anovulatory cycles, and in those with luteal deficiency.

Application to research

Resorption rate. Pregnanediol determinations can be used to control the resorption rate and the duration of effect when administering progesterone.

We injected 100 mg. of progesterone, first in oil solution, then in aqueous suspension, into menopausal women and we compared the daily pregnanediol excretion. It seems that the resorption from the oil solutions follows a more regular pattern, whereas the prolonged effect expected from the crystallized preparation is not very consistent. In 3 patients, the first was given an injection of 200 mg. in oil; the second, 200 mg. in water; and the third, an implantation of two 100-mg. tablets. Pregnanediol recovery was surprisingly high in the case of implantation—77 per cent as compared to the crystal suspension and the oil solution which gave 19 per cent and 11 per cent recovery, respectively. These results may be accidental and, having been obtained from 3 different patients, are not strictly comparable; but it may be interesting to follow this line in further metabolism studies.

Vitamin E. On the whole, the present knowledge of progesterone metabolism is rather meager. We were able to make a contribution concerning the effect of vitamin E (10). In menopausal cancer patients, the pregnanediol yield following progesterone injection was appreciably higher after vitamin E treatment than without it. This result may be interpreted as a shift in progesterone metabolism provoked by the anti-oxidative effect of the vitamin. Marrian (15) could not reproduce these results with normal subjects under otherwise identical conditions, with the only exception that he did not control the vitamin E blood level. Recently, Seegar-Jones and her co-workers (28) did not find any significant difference in pregnanediol excretion between untreated and vitamin E-treated normal women during the menstrual cycle; the vitamin E blood level did not change under treatment. Much further work will be necessary to elucidate these divergent results.

Natural estrogens. We have discussed the pregnanediol decrease accompanying stilbestrol treatment in pregnancy. It seems reasonable to ask whether perhaps the natural estrogens would produce a similar effect. In 1 castrated woman, 39 years old, we recovered 10.5 mg. of pregnanediol after the injection of 100 mg. of progesterone. Then we gave her the customary estradiol doses needed to produce endometrial proliferation, and repeated the progesterone injection. The pregnanediol yield of 6.8 mg. would seem to indicate that the presence of a proliferated endometrium, if anything, *decreases* the progesterone percentage transformed into pregnanediol after a single injection.

Rheumatoid arthritis. Recently Sommerville and co-workers (3) applied the same type of metabolism study to some cases of rheumatoid arthritis. They obtained the very interesting result that appreciably more pregnanediol was excreted by these patients than by normal men and women. We do not know exactly what this means, but believe that these important findings open up a new line of clinical investigation. It may very well be

that some other diseases are associated not only with hormonal deficiency but also with alterations of steroid metabolism.

SUMMARY AND CONCLUSIONS

The scientific interest of pregnanediol determinations lies in the fact that they lend themselves easily to studies on metabolism, rate of resorption, and on duration of effect following progesterone administration.

Their clinical interest is twofold. In pregnancy complications, they are a diagnostic aid for the detection of luteal and placental deficiency; but we must always remember that the trend in a series of determinations is much more informative than single results, which may fall frequently within the wide range of normal variability. In cases of sterility, in abnormal uterine bleeding, and in endocrine disorders, pregnanediol determination provides a reliable test for the presence of a functional corpus luteum. The presence of pregnanediol excludes gross sex hormone disorders of ovarian or pituitary origin.

Acknowledgment

The experimental results reported in this paper have been obtained in collaboration with Dr. R. Borth, who also helped in the preparation of the manuscript.

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SECRETORY AND DECIDUAL CHANGES INDUCED IN THE ENDOMETRIUM OF OOPHORECTOMIZED WOMEN BY ADMINISTRATION OF ESTROGEN AND PROGESTERONE

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THERE have been relatively few studies of the histologic response of the human endometrium to progesterone and only one or two in which serial biopsies have been employed to demonstrate the endometrial histology before and during progesterone therapy. Earlier studies of Neustaedter (1) and of Elden (2) using total doses of 12 to 60 rabbit units of progestin, produced largely negative results. Kaufmann (3) used corpus luteum extracts in doses of 10 rabbit units daily for eight days and reported the induction of secretory changes in the uterus of a castrate woman. Salmon, Walter and Geist (4) reported 5 patients treated with oral anhydro-hydroxy-progesterone (pregneninolone) in whom, even though there was only one biopsy, a good secretory endometrium seemed to have been induced after eight days. These women had been given estrogen for ten weeks prior to the pregneninolone treatment. Ryden (5) in a comparative study of several estrogens, terminated some of his study intervals by giving a total dose of 25 to 65 mg. of progesterone during the last week. He had serial endometrial biopsies to control his observations but he obtained only incomplete secretory changes. Szarka (6) reported the recovery of a decidual cast after twenty-eight days of progesterone therapy in an ovariectomized woman. She had received 5 mg. of estradiol benzoate daily in the month prior to and during the period of progesterone injections. The progesterone dosage was started at 10 mg. on alternate days and then increased to 20 mg. daily for three weeks. The estrogen dosage was decreased and discontinued during the last week of progesterone; then bleeding started and the endometrium was shed as a decidual cast. This indicated that the estrogen was equally as important as the progesterone in maintaining the decidua.

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In an earlier report (7), we have shown that when the function of the corpus luteum is prolonged by large doses of chorionic gonadotropin, the endometrium undergoes definite decidual changes. These findings suggested that if these secretory and decidual changes could be duplicated by administering estrogen and progesterone they would afford some indication of the amount of progesterone elaborated by the human corpus luteum in a normal cycle and in early pregnancy. The present report summarizes the results of preliminary experiments designed to determine the amount of estrogen and progesterone necessary to bring about proliferative and secretory changes of the endometrium in castrate women and subsequently its conversion into decidual tisue.

MATERIALS AND METHODS

The study was conducted on 4 young female inmates of a mental hospital, whose history revealed they had been subjected to bilateral oophorectomy. In each experiment, the 4 patients were started on treatment at the same time. Endometrial biopsy specimens were obtained each week in order to determine the sequence of changes during treatment. Several forms of estrogen were used.[1] The progesterone[1] was given in oily solution, except in two experiments in which it was given dissolved in oil and 5 per cent wax. For the larger doses (75 and 100 mg. per day) crystalline progesterone[1] was given dissolved in sesame oil (75 mg./cc.). Since this was a supersaturated solution, it had to be warmed before injection because the progesterone crystallized out thirty or forty minutes after cooling to room temperature.

EXPERIMENTAL

In the first experiment, estrogen (Menagen), 20,000 I.U. per day, was given orally. This dose proved to be too small, since 5 of the endometrial samples taken during the first two weeks of treatment showed atrophy or were inadequate for interpretation (Chart 1). In 1 patient (N.W.) there was some proliferative response which subsequently was partially converted to a secretory pattern by 5 mg. of progesterone daily for one week. Raising the dose of progesterone to 20 mg. per day and giving estrone intramuscularly for one week brought about secretory changes, but 3 of the women started to bleed while under treatment.

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[1] The estrone in oil (Theelin), the aqueous suspension of estrone (Theelin) and the mixed estrogen (Menagen) were furnished by Parke, Davis & Co. The estradiol benzoate (1034 EX) and the aqueous suspension of estrogens (RB 134–44) were provided by Lakeside Laboratories. The progesterone (50 mg./cc. in oil and 5% wax, and oily solutions of 5.0 and 20.0 mg./cc. (153441)) and crystalline progesterone (153445) were supplied by Ayerst, McKenna & Harrison, Ltd.

In the next experiment, a dose of 2 mg. of estrone was given intramuscularly on alternate days for one week and then 2 mg. daily (Chart 2). On this dose schedule, 5 mg. of progesterone during the second week induced

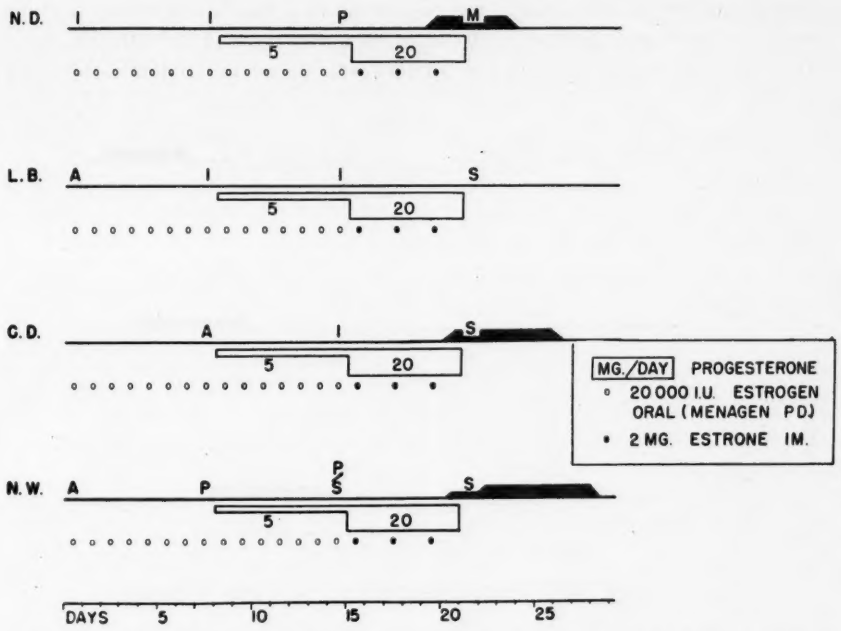

CHART 1. Graphic representation of the endometrial changes found after one week of therapy with mixed estrogen (Menagen), 20,000 I.U. daily; mixed estrogen together with progesterone, 5 mg. daily for one week; and then an aqueous suspension of estrone (Theelin), 2 mg. on alternate days together with 20 mg. of progesterone daily for one week. Menstrual bleeding started during the experiment in 3 instances.

In this and subsequent charts, the letters above the continuous line indicate endometrial biopsies at intervals of one week; **A**—denotes an atrophic endometrium, **R**—Resting, **P**—proliferative, **S**—secretory, **D**—decidual, and **I**—inadequate for interpretation. **M**—denotes a menstrual endometrium that has distintegrated and lost any characteristics of either the proliferative or the secretory phase. The estrogen treatment is indicated by dots or circles and the dosage for each symbol is given on each graph. The number in the open bar indicates the daily dose of progesterone in milligrams. Intervals of menstrual bleeding are indicated by wide black bars.

early secretory changes (subnuclear vacuoles). Menstrual bleeding appeared in 2 of the women during the daily administration of 20 mg. of progesterone, even though secretory changes were evident in the endometria.

In the third experiment, 1 mg. of estrone daily for one week induced good proliferative changes in the endometrium (Chart 3, Fig. 1). Progesterone, 5 mg. daily for the next week, together with the estrone, induced early secretory changes (subnuclear vacuoles, (Fig. 2). Increasing the daily dose of progesterone to 50 mg. and that of estrone to 2 mg. brought about more complete secretory changes (Fig. 3). The glandular epithelium was low-cuboidal with some tufting; the stroma was loose and edematous with

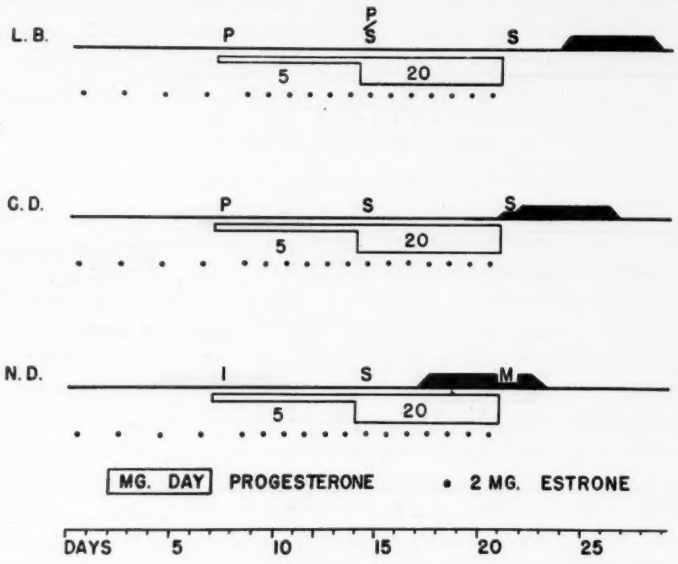

CHART 2. Graphic representation of the endometrial changes found after one week's treatment with an aqueous suspension of estrone (Theelin), 2 mg. per day on alternate days; after estrone 2 mg. daily and progesterone 5 mg. daily for a week; and after estrone together with 20 mg. of progesterone daily for one week. Menstrual bleeding occurred during the last week in 1 case (N.D.) and started at the last day of treatment in another (C.D.).

some predecidual changes. In no instance did menstruation start during treatment.

When estradiol benzoate, 1 mg. per day, was used as the estrogen, a proliferative response was uniformly obtained in the first week (Chart 4). Progesterone, 10 mg. daily for the next week, induced early secretory changes (subnuclear vacuoles in the glandular epithelium and loose edematous stromal tissue). Increasing the dose of estradiol benzoate to 1.5 mg. daily and giving four injections of progesterone (50 mg. each) in the following week produced secretory endometria with definite decidual changes in

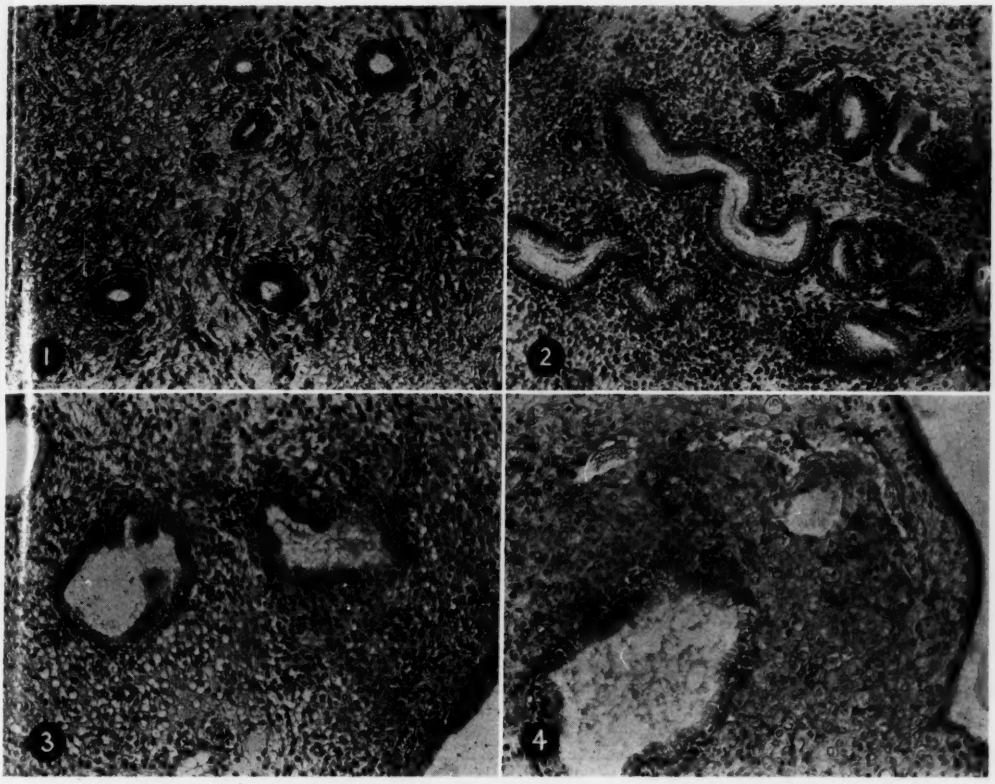

FIG. 1. Proliferative endometrium obtained after treatment for one week with estrone, 1 mg. per day. Second biopsy (P) N.W., Chart 3.

FIG. 2. Early secretory endometrium with subnuclear vacuoles in glandular epithelium and loose edematous stroma obtained after two weeks' treatment with estrone, 1 mg. per day, and one week's treatment with progesterone, 5 mg. per day. Third biopsy (S) N.W., Chart 3.

FIG. 3. Well-developed secretory endometrium with tufting of the glandular epithelium and basal nuclei. Some leukocytic infiltration of the stroma, which is quite edematous. A daily dose of 1 mg. of estrone was given for the first week; a daily dose of 5 mg. of progesterone was given in addition to the estrone in the second week; and during the third week the dosage was 2 mg. of estrone and 50 mg. of progesterone daily. Fourth biopsy (S) N.W., Chart 3.

FIG. 4. Secretory endometrium with decidual changes in the stroma, obtained after three weeks of estradiol benzoate and two weeks of progesterone therapy. Third biopsy (D/S) N.W., Chart 4.

2 instances (C.D. and N.W., Fig. 4). Bleeding started from two to four days following cessation of progesterone therapy, even though the estrogen injections were continued.

Estrogen (2 mg.) was given intramuscularly on alternate days as an aqueous suspension of crystals (Chart 5). After one week, the endometrium

was typically proliferative with many mitoses in the glandular epithelium and in the stroma (Fig. 5). During the second week 2 of the subjects were then given progesterone, 50 mg. per day, and the other 2 received 75-mg. and 100-mg. daily doses, respectively. After one week of this combined estrogen and progesterone therapy, the endometrium was characterized

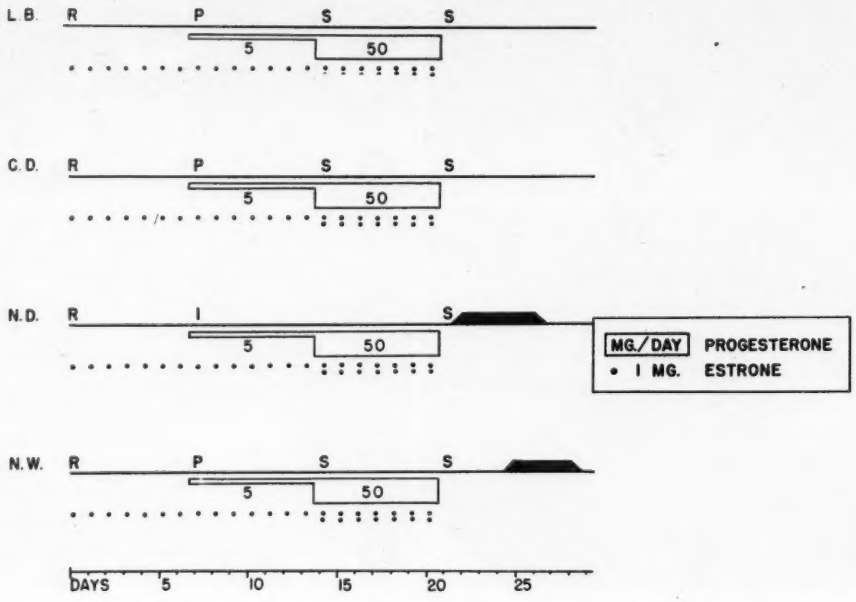

CHART 3. Graphic representation of the endometrial changes found after one week's treatment with estrone (Theelin-in-oil), 1 mg. daily; after 1 mg. of estrone and 5 mg. of progesterone daily for one week; and after 2 mg. of estrone together with 50 mg. of progesterone daily for one week.

by tall glandular epithelium with prominent subnuclear vacuoles (Fig. 6). The stroma was more dense than is normally found when subnuclear vacuoles appear in the endometrium during the normal menstrual cycle. This density of the stroma indicated that the estrogen dosage should probably be larger.

On the seventh day of the study, the supply of progesterone was lost accidentally and treatment was interrupted for two days before the material could be replenished. As a result of this interruption, the 2 women who had been receiving 50 mg. of progesterone daily began to bleed, and treatment was discontinued. Daily injections were resumed on the women getting 75 mg. and 100 mg. per day respectively. At the end of the next

week, the woman receiving 75 mg. per day had started to menstruate, and treatment was discontinued. The endometrium of the patient receiving 100 mg. per day had been converted to a secretory type (the glandular epithelium was tufted and nuclei were located basally (Fig. 7)). The stroma was still fairly compact and not as edematous as in the secretory endo-

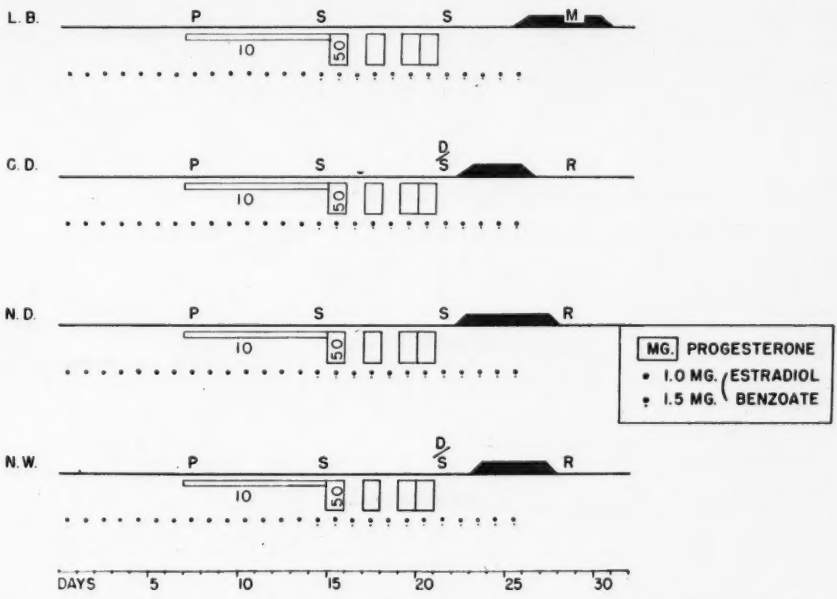

CHART 4. Graphic representation of the endometrial changes found after one week's treatment with estradiol benzoate, 1 mg. daily; after one week of estradiol benzoate and 10 mg. of progesterone daily; and after 1.5 mg. of estradiol benzoate daily and progesterone, four injections of 50 mg. in the third week of the experiment.

metrium of a normal cycle. In an effort to conserve the remaining progesterone, the daily dose was reduced to 80 mg. for the next four days. The endometrial biopsy specimen obtained on the third day after cessation of therapy revealed predecidual changes in the stroma, with definite venous sinuses and a rather marked leukocytic infiltration (Fig. 8). The glandular epithelium was low-cuboidal and the general histologic character was that of premenstrual endometrium.

DISCUSSION

These experiments demonstrate the production of several phases of endometrial morphology.

1. There seems to be a definite time lag in the sequence of changes in the glandular epithelium. Subnuclear vacuoles were present after one week of treatment with progesterone, whether the dose was 5, 50, or 100 mg. per day. Tufting of the epithelium and basal alignment of the nuclei occurred during the second week of treatment, but the decidual change in

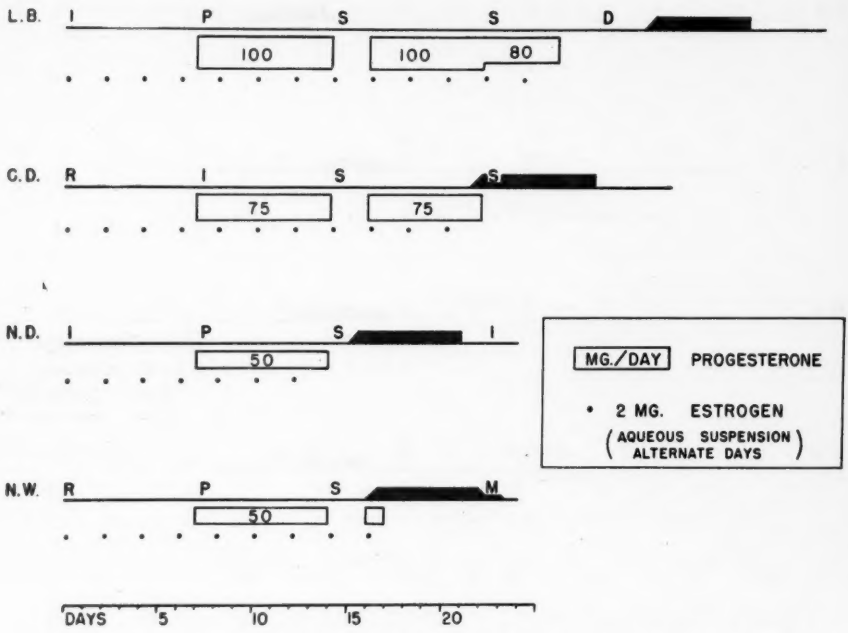

CHART 5. Graphic representation of the endometrial changes induced by treatment with an aqueous suspension of mixed estrogens (Lakeside), 2 mg. on alternate days for one week, and then continued together with large doses of progesterone, 50, 75, and 100 mg. daily. The discontinuity of the progesterone therapy was caused by an accidental loss of the progesterone and there was a two-day interval before the supply was replenished.

the stroma was not evident until the end of the second week. Further experiments in which biopsy specimens are obtained more frequently, or three or four days after a change in dosage, would afford better increments of timing in the sequence of histologic changes. Bickers claims full progestational changes after five days of progesterone treatment (8). In no instance have we succeeded in producing any more than the earliest signs of secretory change in seven days, with the doses used in this study.

2. As a corollary to the first statement, an ascending dose schedule for

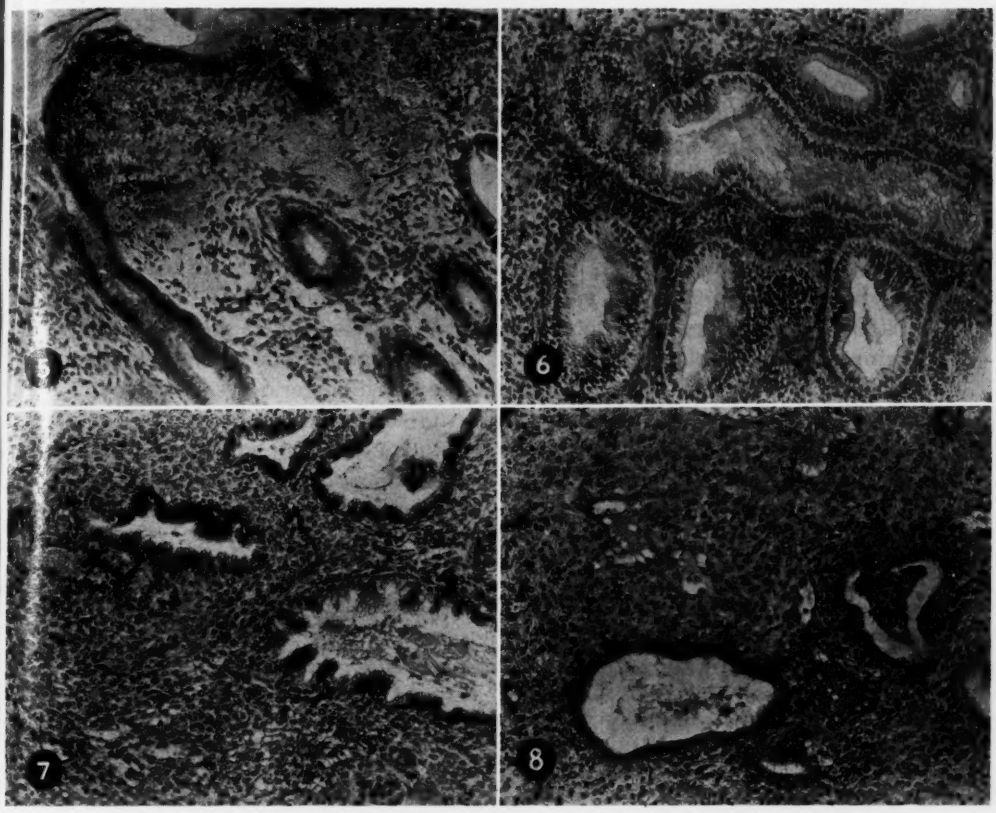

FIG. 5. Proliferative endometrium with mitoses evident in glandular epithelium, obtained after treatment with aqueous suspension of mixed estrogens (Lakeside), 2 mg. on alternate days for one week. Second biopsy (P), L.B., Chart 5.

FIG. 6. Early secretory endometrium, showing tall glandular epithelium with subnuclear vacuoles. Specimen obtained after two weeks' treatment with aqueous suspension of mixed estrogens (Lakeside), 2 mg. on alternate days, and progesterone 100 mg. daily for one week. Third biopsy (S) L.B., Chart 5.

FIG. 7. Secretory endometrium, showing tufting of glandular epithelium and basal nuclei. Specimen obtained after three weeks' treatment with estrogen, and two weeks' treatment with progesterone, 100 mg. daily (except for two days' interruption). Fourth biopsy (S) L.B., Chart 5.

FIG. 8. Secretory endometrium with premenstrual changes (low-cuboidal glandular epithelium, prominent venous sinuses and leukocytic infiltration) obtained four weeks after initiation of estrogen therapy and three days after cessation of progesterone, 80 mg. daily, by injection. Fifth biopsy (D) L.B., Chart 5.

progesterone would be equally as effective as a high dosage maintained constantly for the duration of the experiment.

3. Progesterone is metabolized so rapidly that doses of 50 mg. per day

produce no holdover or depot effect for longer than forty-eight hours, when administered in oily solution.

4. With the doses of estrogen used in these experiments, 20 mg. of progesterone daily for one week was usually insufficient to maintain the endometrium and prevent the onset of menstruation. Allen (9) and Masters and Magallon (10) have noted that patients may bleed during progesterone therapy (10 mg. per day) if it is continued longer than ten days. Szarka used 20 mg. of progesterone per day combined with 5 mg. of estradiol benzoate and no bleeding was experienced during two weeks of this regimen; but bleeding did occur when the estrogen was discontinued.

These preliminary experiments show that further studies are necessary before optimal replacement doses of estrogen and progesterone can be determined for women. The apparent advantage of larger doses of estrogen in Szarka's report and in our experiments suggests that large doses of estrogen may be beneficially combined with progesterone in clinical practice. The apparent beneficial results of large doses of estrogen in cases of threatened abortion may be due to an enhancement of the action of the progesterone that is present.

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THE HYPOGLYCEMIC AND EOSINOPENIC RESPONSE TO INSULIN:

A TEST FOR PITUITARY-ADRENAL INSUFFICIENCY

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THE relationship between the activity of the adrenal cortex and the number of circulating eosinophils has been amply demonstrated. Forsham *et al.* (1) have shown that, in normal humans, the administration of ACTH (which stimulates the adrenal cortex) and compound E or compound F (active corticoids formed by the adrenal cortex) causes an eosinopenia. In addition, Recant *et al.* (2) showed that injections of 0.3 cc. of epinephrine subcutaneously produced a marked drop in eosinophils after four hours, averaging 63 per cent, with a range between 40 and 80 per cent, whereas controls injected with saline did not show this effect. In patients with adrenal insufficiency, the fall in eosinophil count in response to adrenalin was less than 40 per cent.

In 1941, Fraser, Albright and Smith (3) showed that insulin, 0.1 unit per kilo intravenously, produced a response in normal subjects characterized by a maximum drop in blood sugar at about 30 minutes and by a return to the fasting blood sugar level within 90 to 120 minutes. Patients with adrenal and pituitary insufficiency showed increased sensitivity to insulin and also a failure of blood sugar to rise to the pre-test levels within 90 to 120 minutes. These observers suggested the use of 0.05 unit of insulin per kilo in such patients in order to prevent a serious insulin reaction. Nevertheless, the extreme sensitivity of certain patients suffering from adrenal or pituitary insufficiency has discouraged many from using this test.

In 1948, Laragh and Almy (4), following the intravenous administration of insulin in dosages of 0.1 unit per kilo, found an average drop of 57 per cent in blood eosinophils. In control glucose insulin tolerance tests in which the blood sugar level remained constant, no change in blood eosinophils was produced. They found no significant fall in eosinophils in 4 cases of adrenal insufficiency and 2 cases of pituitary insufficiency.

It was the purpose of this investigation to determine the answers to the following questions: 1. How consistently is there a significant decrease in

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eosinophils after the injection of adrenalin or insulin in convalescing patients who have no clinical manifestations of adrenocortical insufficiency? 2. Is there a critical level of hypoglycemia after the injection of insulin, which is regularly associated with a significant fall in eosinophils? 3. Does feeding shortly after hypoglycemia has been attained, inhibit the eosinophil decrease? 4. Do patients with adrenal and pituitary insufficiency respond differently from normal subjects? If the answers to the last three questions were in the affirmative, then in one test adrenocortical function

TABLE 1. CONTROL PATIENTS' FAILURE TO RESPOND TO INSULIN

Diagnosis	Age (yrs.)	Sex	Insulin test		Adrenalin test	
			Initial eosinophil count per cu. mm.	Change in eosinophils %	Initial eosinophil count per cu. mm.	Change in eosinophils %
Boeck's sarcoid	16	M	406	− 8	350	+10
Subacute bacterial endocarditis	16	F	638	−19	530	−29
Tuberculous peritonitis	22	F	600	−22	940	−74
Infectious mononucleosis	16	F	94	−22	125	−80
Trichinosis	18	M	562	−26	445	−46
Tuberculous adenitis	22	M	306	− 6	306	−40

could be studied by determining, (a) insulin sensitivity, (b) blood sugar rise as a response to hypoglycemia, and (c) eosinophil decrease as a response to hypoglycemia.

METHODS

Control patients. Patients without clinical adrenal or pituitary insufficiency were selected from the medical and surgical wards of the Maimonides Hospital. With a few exceptions the patients were below the age of 55. No severely ill or febrile patient was studied.

Methods and techniques. The blood sugar was determined by the method of Nelson (5). Eosinophil counts were made as described by Forsham (1).

Blood samples, when the cells were not counted immediately, were kept in a refrigerator, and the cell count made within four to six hours.

Regular insulin (Squibb) and epinephrine subtartrate (Winthrop) 1:1000 were used. All insulin and epinephrine came from the same stock.

Patients were fasted overnight and breakfast omitted. Blood samples

TABLE 2. CONTROL PATIENTS' FAILURE FO RESPOND TO ADRENALIN

Diagnosis	Age (yrs.)	Sex	Adrenalin test		Insulin test	
			Initial eosinophil count per cu. mm.	Change in eosinophils %	Initial eosinophil count per cu. mm.	Change in eosinophils %
Boeck's sarcoid	16	M	350	+10	406	− 8
Subacute bacterial endocarditis	16	F	530	−29	638	−19
Multiple sclerosis	38	M	108	−12	150	−79
Tuberculous adenitis	52	F	1003	−32	835	−66
Chronic cholecystitis	38	F	100	−12	169	−79
Psychoneurosis	44	F	575	−28	560	−44
Hydronephrosis	54	F	505	−32	475	−41
Ulcerative colitis	44	F	300	−39	248	−92

for sugar and eosinophil determinations were drawn and the calculated dose of insulin, either 0.05 or 0.1 unit per kilo, was administered intravenously. The epinephrine test was performed with 0.3 cc. of epinephrine injected subcutaneously into the deltoid region. In some instances, complete tolerance tests were done with blood sugars drawn at 20, 40, 60, 90, and 120 minutes; and in other instances, 0 and 30-minute blood samples were taken for sugar determination. Eosinophil counts were performed on blood drawn at 0 and 240 minutes after injection of either insulin or adrenalin.

RESULTS

1. *Comparative eosinopenic response to adrenalin and insulin.* Thirty-one control patients received 0.1 unit of insulin per kilo intravenously. Twenty-five of the 31 had a drop in eosinophils greater than 40 per cent. The data of the remaining 6 negative reactors are shown in Table 1. Four of the 6 negative reactors had a significant drop in eosinophils with the adrenalin test. Two patients responded neither to insulin nor to epinephrine.

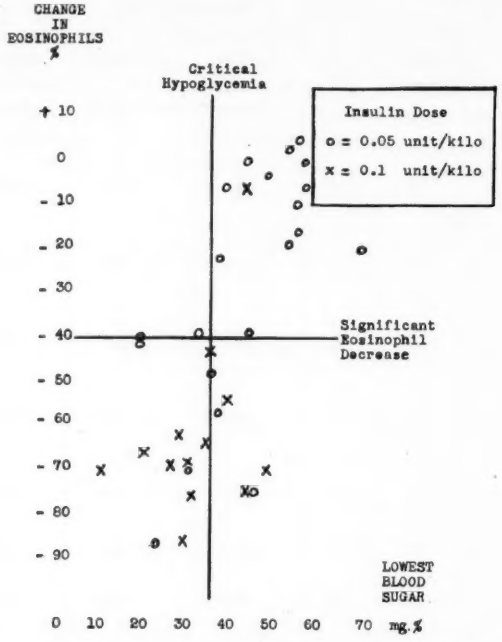

FIG. 1. Correlation between decrease in eosinophils and hypoglycemic level after insulin, in the fasting patient.

Fourteen of 22 patients injected with adrenalin had a drop in eosinophils greater than 40 per cent. Table 2 reveals that 6 of the 8 negative reactors had significant responses to insulin.

2. Correlation between level of hypoglycemia and eosinopenic response. Gershberg and Long (6) demonstrated that in rats, when the blood sugar fell below 40 mg. per cent following insulin injections, the adrenal cortex was stimulated. A series of experiments was performed to determine if a similar correlation existed between depth of blood sugar drop and eosinophil fall in the human. A group of patients received either 0.05 or 0.1 unit of

insulin intravenously per kilo of body weight. These data are presented in Figure 1.

As noted in Table 3, 14 of 15 subjects with blood sugar values below 35 mg. per cent had a drop in eosinophils greater than 40 per cent. However, of 10 patients with blood sugar levels between 35 and 45 mg. per cent only 4 showed more than 40 per cent drop in eosinophils. Only 1 of 10 patients whose blood sugar exceeded 45 mg. per-cent had a significant drop of eosinophils.

TABLE 3. CORRELATION BETWEEN HYPOGLYCÉMIA AND EOSINOPENIC RESPONSE

Lowest blood sugar, mg. %	Drop in eosinophils		Number of patients
	less than 40%	more than 40%	
Below 35	1	14	15
35–45	6	4	10
Above 45	9	1	10

Of the 22 patients who received the 0.05 unit of insulin per kilo, only 5 showed a hypoglycemia below 35 mg. per cent; these 5 patients had significant eosinophil drops. Seven subjects in this group had blood sugar levels between 35 and 45 mg. per cent. Significant eosinopenic responses occurred in only 2 of these 7 subjects in the borderline group.

3. *Effect of feeding on eosinopenic response to hypoglycemia.* Occasionally, patients with adrenal or pituitary insufficiency are so hypersensitive to insulin that it is necessary to administer glucose before the test is completed. In order to determine the effect of feeding, 15 patients who clinically did not have adrenal or pituitary insufficiency received 0.1 unit of insulin per kilo intravenously. Glucose by mouth, as orange juice with breakfast, was given after the 30-minute blood was taken. The results are graphically shown in Figure 2.

Fourteen of the 15 patients had 30-minute blood sugar values of less than 35 mg. per cent. Nine of the 14 patients had less than a 40 per cent drop in eosinophils and the other 5 had more than a 40 per cent drop, as compared to 14 of 15 unfed patients who had eosinophil decreases greater than 40 per cent.

4. *Response of patients with adrenal or pituitary disease to insulin.* The responses to insulin of 4 patients with adrenal insufficiency, and of 1 patient with pituitary insufficiency have been studied. The initial dosage was 0.05 unit of insulin per kilo, and if blood sugar values failed to fall below 35 mg. per cent, a double dose was used.

Two patients with Addison's disease had severe hypoglycemia with no hypoglycemic responsiveness, since they had symptoms of severe hypoglycemia at 45 and 90 minutes after 0.05 unit of insulin per kilo was injected. Because of the severe hypoglycemic symptoms, glucose was administered intravenously; the patients immediately became asymptomatic. They were then fed. As noted in Table 4, there was no fall in eosinophils.

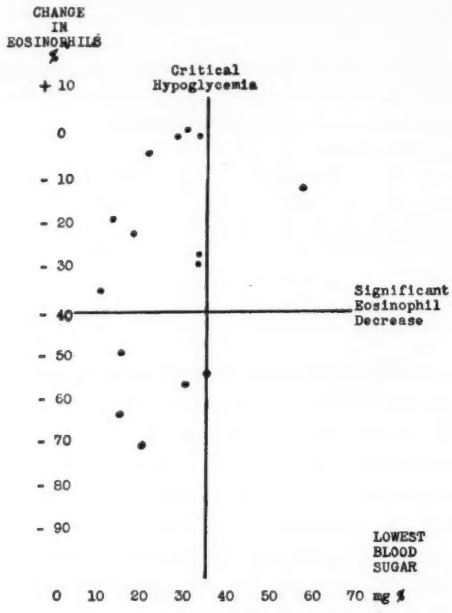

FIG. 2. Correlation between decrease in eosinophils and hypoglycemic level in patients fed 30 minutes after insulin.

However, no conclusion can be drawn from the absence of eosinophil drop, since the patients were fed.

The other 2 patients with adrenal insufficiency showed no hypersensitivity. Both tolerated the dose of 0.1 unit per kilo well. They showed good hypoglycemic responsiveness, and their blood sugar values returned to the pre-test level by 120 minutes. However, they had no significant drop in eosinophils. The fasting eosinophil counts of 156, 75, 130 and 138 were not abnormally high, as has been reported (2). After receiving 0.05 unit of insulin per kilo, a patient with pituitary insufficiency had 30-minute blood sugars of 25 and 30 mg. per cent on two different occasions with no significant drop in eosinophils (Table 4).

In all 3 patients with acromegaly, a hypoglycemia below 35 mg. per cent

failed to develop with a 0.1 unit per kilo dose of insulin. Thus they were insulin resistant. The circulating eosinophils failed to decrease.

DISCUSSION

Forsham and co-workers (1) have demonstrated that, in the normal subject, the injection of either ACTH, cortisone, or compound F is fol-

TABLE 4. RESPONSE TO INSULIN TEST IN ADRENAL OR PITUITARY DISEASE

Diagnosis	Age (yrs.)	Sex	Insulin dose (unit)	Blood sugar (mg. %)				Eosinophil count	
				0 min.	20 min.	40 min.	120 min.	initial, per cu. mm.	change, %
Addison's disease	28	F	.05	55	25	—	55	100	+ 3
			.1	92	8	—	70	75	−25
Addison's disease (tbc.)	44	M	.03	97	—	49	88	69	+16
			.05	91	—	45	66	150	− 2
			.1	60	25	35	60	131	+25
Addison's disease (tbc.)	28	F	.05	70	31	20	shock	156	−16
Addison's disease (tbc.)	27	F	.05	74	—	—	shock	138	+ 6
Hypopituitarism	70	M	.05	88	30	—	—	228	+ 8
			.05	75	25	25	75	300	−27
Acromegaly	38	M	.1	80	40	—	60	140	0
Acromegaly	59	M	.1	70	45	40	70	138	−24
Acromegaly	63	F	.1	112	78	—	92	38	+32

lowed by a sharp fall in circulating eosinophils. Long and Fry (7) reported that the administration of adrenalin directly to the pituitary stimulated the pituitary-adrenocortical axis as manifested by a decrease in adrenal cortex ascorbic acid content. Recant and her co-workers (2) showed conclusively, using the decrease in circulating eosinophils as an indicator, that subcutaneously or intravenously administered adrenalin stimulates the pituitary-adrenocortical system in the rat, dog and human. They noted that, in response to adrenalin injection, a decrease in the eosinophil count of at least 40 per cent was found in all their normal subjects but in only 1

of 11 patients with Addison's disease. Since the decrease of the blood eosinophils in response to adrenalin injection has been advocated as a sensitive test of adrenocortical insufficiency, it was considered important to determine the results of this test in patients convalescing from various diseases. None of these patients demonstrated any clinical evidence of adrenocortical insufficiency. However, since many had received antibiotics during their antecedent illness, the initial circulating eosinophil levels were abnormally high in some of these subjects. It is difficult to know what percentage fall of eosinophils should be considered significant in these patients with high initial counts. Many of our control patients, who responded with eosinophil drops in excess of 40 per cent, had initial eosinophil levels considerably over 400. Therefore, we chose to accept Recant's standards at the risk of having excessive numbers of false failures to respond to adrenalin. Tables 1 and 2 reveal that, despite initial counts over 300, 6 of 8 control patients who failed to respond to one insulin or to one adrenalin injection, did respond to the other of these two stimuli. Eight of 22 patients who had no clinical manifestations of adrenal insufficiency failed to respond to adrenalin with a significant eosinopenia. Thus false positive responses in our convalescing patients were too frequent to be of definitive value in diagnosing adrenal insufficiency.

Pellegrino *et al.* (8) have reported that the injection of 0.3 mg. of adrenalin failed to cause a 40 per cent drop of eosinophils in 3 of 11 hospitalized patients without obvious adrenal disease. Madison (9) noted that 0.3 mg. of adrenalin in normal males failed to cause a 40 per cent decrease of eosinophils in 4 of their 31 experiments.

The hypoglycemia induced by insulin is associated with an increased secretion of adrenalin into the blood. Long and Fry (7) demonstrated that adrenalin increases the activity of the adrenal cortex. Thus it seems probable that insulin activates the adrenal cortex by means of increasing adrenalin secretion.

However, Gellhorn and Frank (10) reported that insulin hypoglycemia induced some increase in adrenocortical secretion even in the demedullated animal. This suggested that insulin might stimulate the adrenal cortex by other means than inducing the secretion of adrenalin. It was, therefore, of interest to determine whether insulin hypoglycemia was more reliable than adrenalin as a stimulus to the adrenal cortex.

Six of 31 patients, receiving an intravenous injection of 0.1 unit of insulin per kilo, failed to show an eosinopenia. Thus the stimulus of insulin or epinephrine in hospital subjects convalescing from acute illness or suffering chronic illness may not be sufficient in all instances to excite the adrenal cortex. However, the data presented suggest that insulin is as active as epinephrine in causing an eosinopenic response.

Gershberg and Long (6) have shown that the decrease in the rat's adrenal ascorbic acid content was initiated by a critical hypoglycemic blood level rather than by a definite dose of insulin. In rats, the critical level of blood glucose is 40 mg. per cent. Therefore, further studies were made to determine if there was a critical level of hypoglycemia in man which would consistently initiate a decrease in eosinophils. It was demonstrated that blood sugar levels at or below 35 mg. per cent were associated with definite decreases in eosinophils in 14 of 15 patients. Thus in our patients, hypoglycemia of 35 mg. per cent or less seemed to be a more reliable adrenocortical stimulus than the injection of adrenalin.

TABLE 5. RESPONSE TO INSULIN TEST IN CONTROL PATIENTS AND IN PATIENTS WITH PITUITARY-ADRENAL DISEASE

	Insulin sensitivity	Response to hypoglycemia	
		Return of blood glucose to pre-test level	Circulating eosinophil decrease
Control series	Normal	Complete	Significant
Addison's disease			
(a) severe carbohydrate deficiency	Hypersensitive	Delayed and incomplete	Insignificant (?)
(b) mild carbohydrate deficiency	Sensitive	Complete	Insignificant
Hypopituitarism	Sensitive	Complete	Insignificant
Acromegaly	Insensitive	Complete	Not determined

Since patients with adrenal insufficiency are often very sensitive to insulin, it has been necessary to terminate the insulin tolerance test in such cases by injecting or feeding glucose. Therefore, it was of importance to determine if feeding would inhibit the eosinophil decrease, once a definite hypoglycemia had been established by the injection of insulin. Only 5 of 14 patients who had hypoglycemia of 35 mg. per cent or less showed a fall in eosinophils when they were fed 30 minutes after the intravenous injection of insulin. This demonstrated that feeding will prevent eosinophil fall in a sufficient number of patients to make invalid the data of eosinophil fall in patients who have to be fed because of the development of severe hypoglycemia.

As noted in Tables 4 and 5, the response of patients with Addison's dis-

ease to insulin has fallen into two groups in this small series. The patients in the insulin-sensitive group had such severe symptoms, at 45 and 90 minutes after insulin injection, that the test had to be terminated. Although the eosinophil counts of these patients did not fall, these data signify little, since the patients were fed during the test. Since none of our normal patients had a severe reaction with 0.05 unit of insulin per kilo, the severity of the reaction with this small dose suggests that these patients were insulin-hypersensitive. The other 2 patients with Addison's disease were able to tolerate full doses of insulin, 0.1 unit per kilo, without severe insulin reactions clinically. Their blood sugar values returned to the pretest level at 120 minutes. All had blood sugar levels below 35 mg. per cent. None had a significant fall in eosinophils. All of these 4 Addisonian patients had excessive pigmentation, hypotension, strongly positive results with the Robinson-Power-Kepler water excretion tests, and low 17-ketosteroid excretions.

It would be of interest, in the study of patients with adrenal insufficiency, to correlate the level of urinary 11-oxysteroid excretion with the response to insulin. If marked insulin sensitivity, as demonstrated by severe hypoglycemic response to small doses of insulin, is a measure of the lack of adrenal "S" hormone, then one might expect it to be associated with marked decrease in the 11-oxysteroid excretion. If this hypothesis were valid, the other patients with Addison's disease, in whom the response to insulin is demonstrated only by a lack of eosinophil fall, might have only moderate, or no, decrease of the 11-oxysteroid excretion.

To prevent severe hypoglycemia, it is proposed that 0.03 unit of regular insulin per kilo be used as a preliminary dose. When performing this test on patients with adrenal insufficiency, a physician must be readily available at all times and a syringe containing 50 cc. of 50 per cent glucose should be ready. With these precautions, the danger to the patient can be minimized. In patients with arteriosclerotic cardiac or cerebral disease, hypoglycemia must be avoided and this test should not be made.

Four patients with pituitary dysfunction were studied. The single patient with hypopituitarism demonstrated no insulin hypersensitivity but there was no eosinophil fall. Thus the response was similar to that of the less insulin-sensitive Addisonian patients. The 3 acromegalic patients all demonstrated insulin resistance. In none of these was there sufficient hypoglycemia, 35 mg. per cent or less, to initiate an eosinophil drop. Unfortunately, in these insulin-resistant acromegalic patients we failed to determine if hypoglycemia below 35 mg. per cent would be caused by increasing the doses of insulin. Until this critical hypoglycemic level is reached, the eosinophil response to pituitary adrenal stimulation cannot be determined in these patients.

SUMMARY AND CONCLUSION

1. A study has been made of the stimulation of the adrenal cortex by either adrenalin or insulin, as measured by a decrease in the eosinophils. The subjects were patients with either Addison's disease, hypopituitarism, or acromegaly, together with control patients convalescing from other nonendocrine disorders.

2. Eight of 22 control patients failed to show a significant decrease in eosinophils after receiving adrenalin.

3. In the convalescent control patients, insulin was as effective as adrenalin in causing eosinopenia. The hypoglycemic level, rather than the dose of insulin, is the critical factor in initiating a decrease in eosinophils. A hypoglycemic level of 35 mg. per cent was usually associated with a definite decrease in eosinophils. Blood sugar levels between 35 mg. per cent and 45 mg. per cent were associated with variable eosinopenic responses. Hypoglycemic levels of 35 mg. per cent or less were not regularly associated with eosinopenia if the patient was fed as soon as hypoglycemia developed.

4. Two patients with Addison's disease demonstrated insulin sensitivity and retarded blood glucose rise in response to hypoglycemia. Two other patients with Addison's disease demonstrated no insulin hypersensitivity and had a normal blood glucose rise in response to hypoglycemia. There was no eosinophil drop in any of these 4 patients, despite hypoglycemia below 35 mg. per cent.

Insulin hypersensitivity and hypoglycemia unresponsiveness may indicate more severe adrenal disorder of carbohydrate function than the mere lack of eosinopenic response in the presence of normal insulin sensitivity and hypoglycemic responsiveness.

5. One patient with hypopituitarism failed to have an eosinopenic response to hypoglycemic levels of 25 and 30 mg. per cent. Three acromegalic patients showed insulin resistance.

6. It is concluded that the blood sugar and eosinophil response to insulin hypoglycemia may be used as a test of adrenocortical insufficiency in man.

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UPTAKE OF RADIOACTIVE IODINE AFTER INTRAVENOUS ADMINISTRATION OF TRACER DOSES*

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AFTER oral administration of radioactive iodine, the uptake of the tracer by the thyroid gland is abnormally high in most cases of untreated hyperthyroidism (1, 2, 3). However, a significant overlap exists between the normal range of uptake and the range in hyperthyroidism (4, 5); cases with uptakes in the range of overlap must be considered borderline. Since it is in these borderline cases that the clinician has most difficulty in establishing the correct diagnosis, any safe procedure which would reduce the area of overlap would be welcome. It seemed desirable to determine if this purpose might be accomplished by administering the tracer intravenously, thereby eliminating the variables of rate and amount of gastrointestinal absorption. It was also hoped that the intravenous method would offer the additional advantages of rapidity and convenience.

METHODS

Carrier-free radioactive iodine, I^{131}, was prepared for intravenous use by dilution with sterile physiologic saline in sterilized, rubber-capped bottles. The bottles were then placed in a boiling water bath for ten minutes with a 25-gauge needle through the stopper for an air vent, after which their contents were standardized against a standard solution of radioiodine. The dilution was such that some 5 or 10 cc. carried the desired test dose of 40 to 100 microcuries. This was given rapidly by vein. Readings with a shielded Geiger counter 10 centimeters from the thyroid region were begun immediately and repeated every ten or fifteen minutes for an hour, and again at twenty-four hours. Immediately after each reading over the neck, a reading was taken at the same distance at mid-thigh level and applied as a background correction by subtraction from the neck reading. By comparing the net count with the radioactivity of a standard solution, the quantity of I^{131} in the thyroid was calculated and recorded in terms of per cent of the administered dose.

No attempt was made to analyze the diets of the experimental subjects

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for iodine content, and the test was usually performed in the postabsorptive state.

Basal metabolic rates were obtained on nearly all subjects, usually a few days before the uptake test. In some instances serum protein-bound iodine determinations were performed in another laboratory, utilizing Salter and McKay's modification of the method of Sandell and Kolthoff (6).

MATERIAL

The experimental subjects included: 1) a control group of 19 adults, 6 males and 13 females, who were considered to be clinically euthyroid, had no goiter, no history of goiter or thyroid disease, and who had never been given iodine, desiccated thyroid or thiouracil derivatives; 2) 18 patients, 1 male and 17 females, with clinically unmistakable, untreated hyperthyroidism as evidenced by the presence of characteristic symptoms and obvious physical signs, including an enlarged thyroid gland; 3) 8 patients, all female, with a clinical diagnosis of nontoxic goiter; 4) 3 female subjects with a clinical diagnosis of probable hyperthyroidism, who had palpable thyroid glands and many symptoms suggesting a large element of anxiety, but only equivocal signs of hyperthyroidism; 5) 1 female with classical myxedema and a basal metabolic rate of −36 per cent.

RESULTS

Euthyroidism. In the 19 control subjects the uptake in the thyroid at the end of one hour did not exceed 11 per cent nor fall below 4 per cent (Fig. 1). Within statistical uncertainty of counting, these cases reached the plateau of uptake within the first half hour.

Hyperthyroidism. The uptake in the 18 patients with hyperthyroidism exceeded the normal range in every instance. Uptake at the end of one hour varied from 18 per cent to 72 per cent (Fig. 1). The separation between the group of euthyroid controls and the group of hyperthyroid patients was apparent as early as twenty minutes after administration of the tracer and as early as five minutes for those cases with the highest uptakes (Fig. 1). Rate of uptake was greatest during the first half hour; uptake continued to increase measurably but more slowly during the second half hour. Although there are too few to make certain, yet there is a suggestion that these hyperthyroid cases fall into two groups—one group of 5 with a very rapid initial uptake of 35 to 45 per cent in the first quarter hour, with subsequent marked slowing of the rate of uptake; and another group of the remaining cases showing a lower total 1-hour uptake but a much more even rate throughout the first sixty minutes.

Nontoxic goiter. The 1-hour uptake in the 8 cases of nontoxic goiter was in the normal range (Fig. 2).

Probable hyperthyroidism. In the 3 individuals with probable hyperthyroidism, uptake in all cases exceeded the normal range but tended to be lower than the range for the patients with more obvious signs and symptoms of thyrotoxicosis. The 1-hour uptake in this group varied from 13 per cent to 17 per cent (Fig. 2).

Myxedema. In the single case of myxedema studied, uptake was 2 per

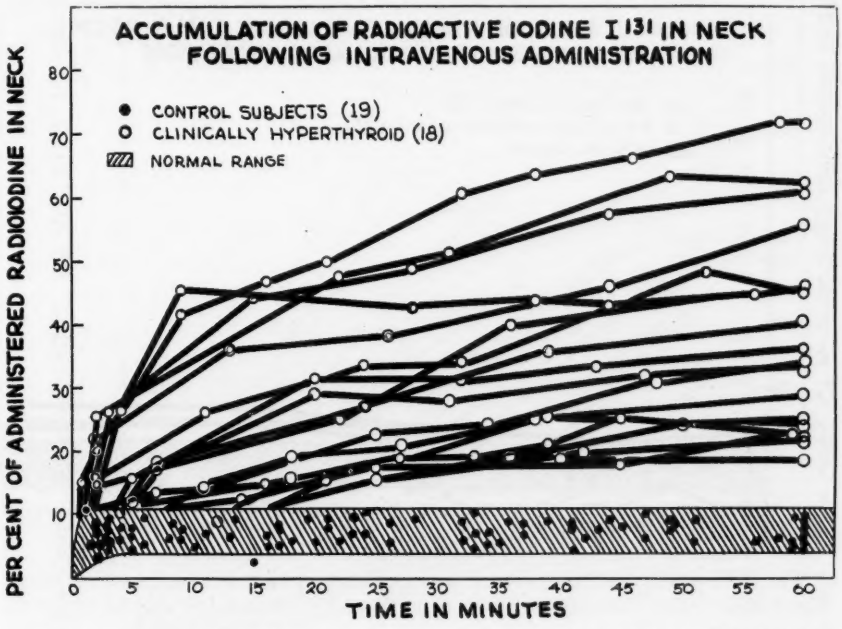

FIG. 1. Radioactivity in the neck after intravenous injection of I^{131}, 100 microcuries or less, plotted as per cent of injected dose. In clinically euthyroid cases, the level did not rise above 11 per cent. In those cases clinically classed as hyperthyroid, the level attained at one hour was above 18 per cent. Those attaining the highest level at one hour showed the most rapid rise in the first fifteen minutes, proportionately as well as absolutely.

cent at the end of one hour and 5 per cent at the end of twenty-four hours.

Comparison of 1-hour and 24-hour uptake. When the 1-hour uptake value is compared with the 24-hour value, several interesting findings will be noted (Fig. 3). Whereas there was good differentiation between hyperthyroid subjects and controls at one hour, there was definite overlapping of results at twenty-four hours. Usually the uptake at twenty-four hours was greater than at one hour for both euthyroid and hyperthyroid individuals. Notable exceptions are the 2 hyperthyroid patients with 1-hour uptakes in

the range of 60 to 80 per cent. Both patients had an uptake higher at one hour than at twenty-four hours. Indeed, in 1 of these patients the 24-hour value was nearly in the normal range. A similar but less pronounced tendency was observed in 2 other hyperthyroid patients with 1-hour uptakes in the range of 50 to 60 per cent. The significance of this observation

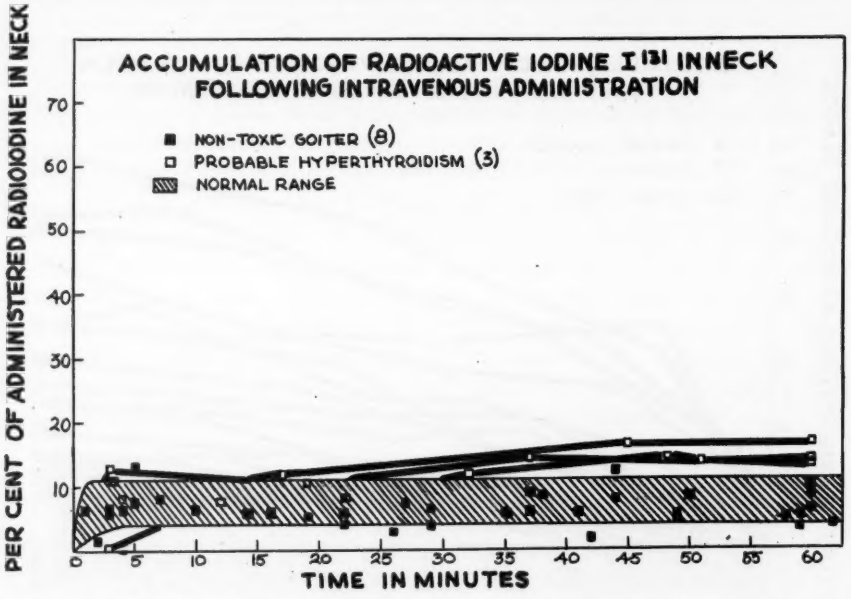

FIG. 2. Clinically nontoxic goiter. Eight cases show uptakes within the normal range. Three cases called clinically probably hyperthyroid show uptakes slightly above the normal range.

will be discussed below. Although it is impossible to establish a definite objective measure of over-all clinical toxicity, it was the impression of the investigator that there was excellent correlation between the 1-hour uptake and the degree of clinical toxicity, whereas correlation with the 24-hour uptake was less satisfactory.

Correlation of 1-hour uptake and basal metabolic rate. The correlation between the 1-hour iodine uptake and the basal metabolic rate is presented graphically in Figure 4. It will be noted that the correlation is excellent for those cases which were clinically hyperthyroid, but there is no correlation for the euthyroid subjects. The latter included a few cases of anxiety state, heart disease, and parkinsonism. It is believed that the elevated basal metabolic rates observed in the euthyroid subjects are not caused by thy-

roid hyperfunction and are to be considered falsely high from this standpoint.

Correlation of 1-hour uptake and serum protein-bound iodine. It will be noted (Fig. 5) that there is fairly good correlation between 1-hour uptake and serum iodine in the group of hyperthyroid patients and poor correla-

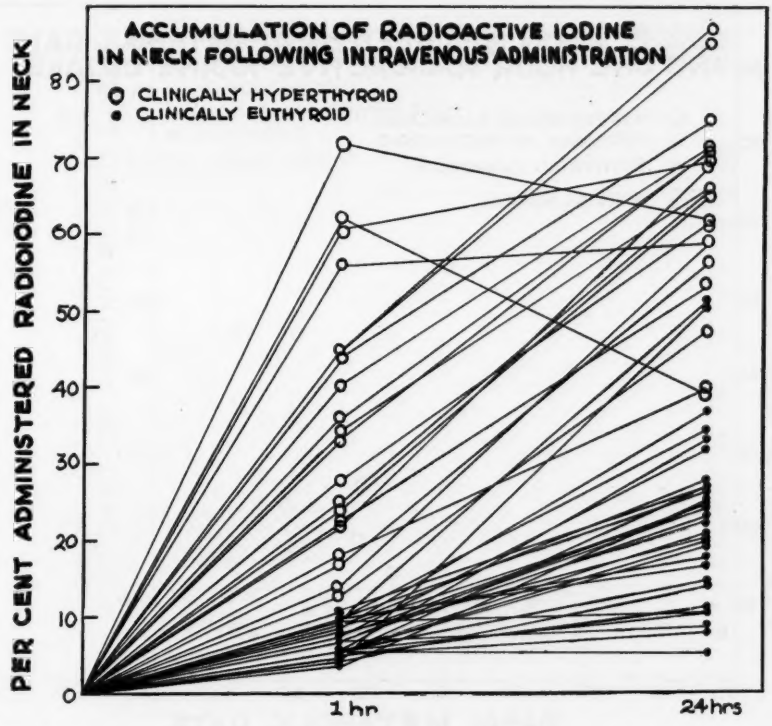

FIG. 3. Two groups of cases, called respectively clinically hyperthyroid and clinically euthyroid, fall into separate ranges of I^{131} uptake as measured one hour after intravenous administration, but show some overlap at twenty-four hours. Note that the 4 cases of highest 1-hour uptake are hardly any higher at twenty-four hours, and in 2 cases actually much lower again.

tion in the euthyroid group. In 3 cases, clinically euthyroid, elevated protein-bound iodine was found without adequate explanation.

Toxicity. No untoward reactions from intravenous administration of the tracer were observed.

DISCUSSION

From a study of the uptake curves in hyperthyroidism following intravenous administration of radioiodine, it is apparent that during the first few

minutes the rate of iodine uptake may be extremely rapid. It seems probable that the initial rapid rate of uptake represents largely trapping of inorganic iodide, whereas later some conversion of iodide into organically bound iodine is also likely. It might be that early measurement after intravenous injection accomplishes somewhat the same thing that Raben and

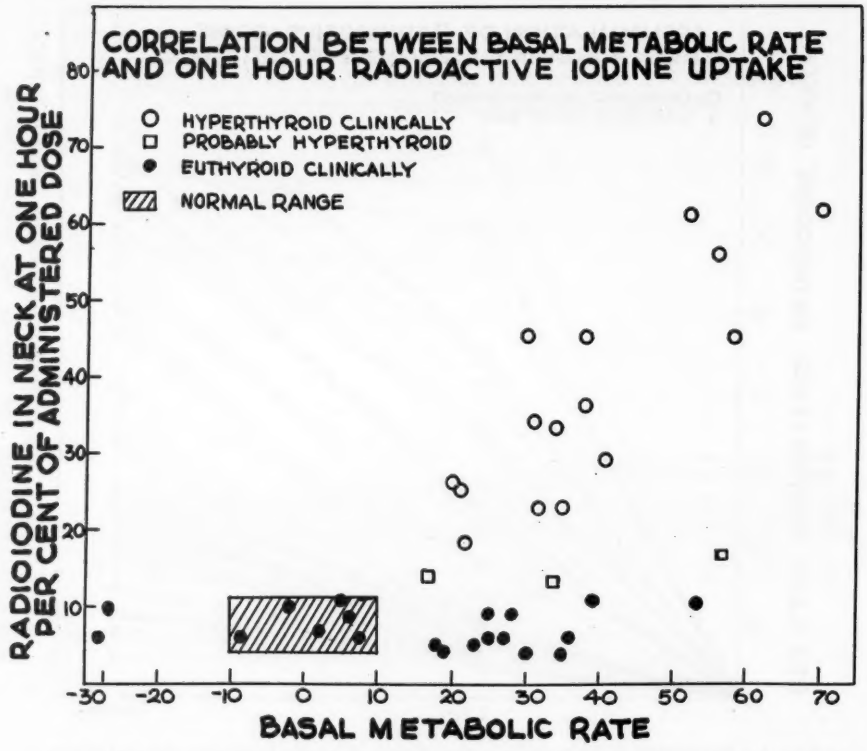

FIG. 4. Basal metabolic rate and per cent 1-hour uptake after intravenous I^{131} are well correlated in cases called clinically hyperthyroid, but show no correlation in euthyroid cases.

Astwood (7) did by giving 1-methyl-2-mercaptoimidazole, namely removal of the complicating factor of organic binding. The authors similarly report a cleaner separation of hyperthyroid from euthyroid cases when only the inorganic iodide uptake is measured. In 2 instances of severe hyperthyroidism reported herein, the 24-hour uptake was less than the 1-hour uptake. Probably in these cases the turnover of tracer was so rapid that by twenty-four hours a significant proportion of the I^{131} had already been converted into thyroid hormone and discharged into the blood stream. This

suggestion finds support from the study of Keating *et al.* (8) who demonstrated that in 3 cases of severe exophthalmic goiter with high basal metabolic rates, the urinary excretion of iodine resembled that of normal subjects and was considerably greater than in other less severe cases of hyperthyroidism. It is important to recognize that rapid turnover of iodine may

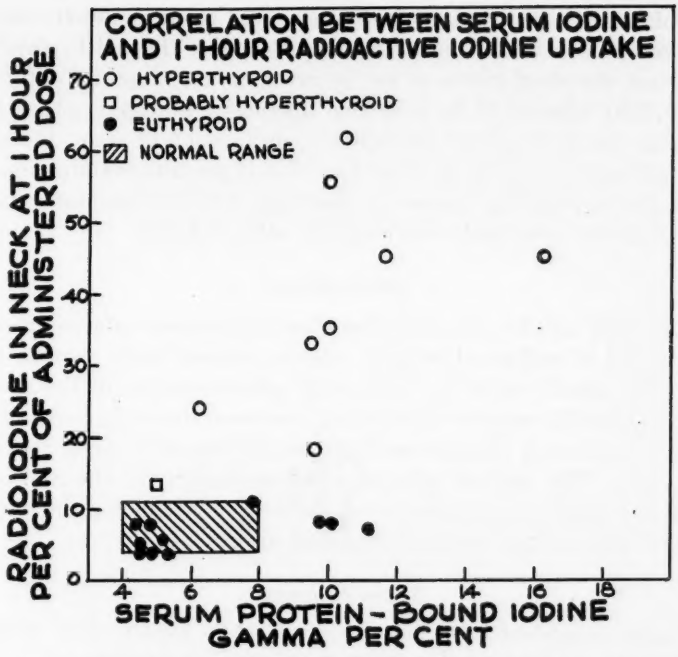

FIG. 5. Serum protein-bound iodine and per cent 1-hour uptake after intravenous I^{131} are fairly well correlated in cases called clinically hyperthyroid.

occur in severe hyperthyroidism, because in such cases the high peak of activity in the neck is evanescent, and the 24-hour measurement may be far into the secondary decline and quite misleading.

The fact that iodine uptake and clinical status do not correlate well with the serum protein-bound iodine level has been pointed out by Williams (10) and by Jaffe and Ottoman (5). In the present study, lack of correlation was observed in 3 euthyroid and in 2 hyperthyroid subjects (Fig. 5).

It was observed that correlation of the 1-hour iodine uptake with the basal metabolic rate was excellent for hyperthyroid subjects (Fig. 4), but that several euthyroid subjects had metabolic rates which were considered falsely high. Falsely high basal metabolic rates were also observed in euthy-

roid individuals by Jaffe and Ottoman (5) and by Bartels (11). The latter demonstrated that pentothal anesthesia reduced the metabolic rate to normal in euthyroid subjects. Anesthesia administered to hyperthyroid subjects, on the other hand, did not materially affect the basal metabolic rate. The data presented in this paper would support the contention that in hyperthyroidism the basal metabolic rate is a reliable measure of the degree of toxicity, but that a reported elevated basal metabolic rate must not be accepted as certain proof of the presence of thyroid hyperactivity.

Although the chief virtue of the intravenous technique of determining iodine uptake appears to be enhanced diagnostic accuracy, other advantages over the oral method are rapidity, elimination of error due to accidental spilling or choking, and the fact that it permits examination of the patient who is vomiting, has severe diarrhea, fistulous communications, or impaired gastro-intestinal absorption for other reasons.

CONCLUSIONS

The thyroid uptake of radioiodine after intravenous administration has been studied in euthyroid subjects and in patients with various thyroid states. The uptake value one hour after administration of the tracer has been found to differentiate satisfactorily between the euthyroid and hyperthyroid state, and it exhibits excellent correlation with the degree of clinical toxicity. The method also offers advantages over the 24-hour oral method in rapidity and in diagnostic accuracy by reducing the overlap in values between normal and hyperthyroid subjects.

Acknowledgments

The author is indebted to Dr. Robert R. Newell of the Department of Radiobiology for his generous aid and counsel in executing and reporting this study.

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LATE RESULTS FROM CONTINUOUS TREATMENT OF THYROTOXICOSIS WITH METHYLTHIOURACIL

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EVER since Astwood (1) in 1943 introduced the employment of antithyroid substances in the clinical treatment of thyrotoxicosis, the discussion has chiefly dealt with the question of whether it might be practicable with these remedies to carry through a conservative treatment of thyrotoxicosis—in brief, to perform "chemical" thyroidectomy with just as good results as may be obtained with surgical thyroidectomy. Now, enough time has elapsed since its introduction that it should be possible to estimate the value of this particular method of treatment, and to obtain statistics regarding the frequency of recurrence.

According to the previous reports from the Medical Department B of the Bispebjerg Hospital (2, 3, 4) as compared to the reports published from other clinics (5, 6, 7, 8), it appears as if after a certain length of time (averaging one year) the methylthiouracil therapy may be discontinued in a number of cases without any particular risk of recurrence. According to these preliminary reports, then, it would appear as if definite recovery from thyrotoxicosis might take place without operative treatment. In this paper a new account is given of the available patient material.

CLINICAL MATERIAL

The present material comprises 244 patients with thyrotoxicosis admitted to the Medical Department B of the Bispebjerg Hospital from February 1944 to August 1949. All these patients have been treated with methylthiouracil continuously. When the methylthiouracil therapy was adopted in this department, the principle was established at once that all cases of thyrotoxicosis were to be treated with methylthiouracil as the only form of treatment. It seemed rational to test the remedy thoroughly according to these principles and then later, through a revision, to establish more clearly the indications and possible contraindications for continuous treatment.

Of the total material, 90 per cent were women and 10 per cent, men. The youngest patient was 15 years old at the institution of treatment, and the oldest was 78. The average age for the male patients was 46.8 years and for the females, 44.3 years.

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All these patients presented clear-cut cases of thyrotoxicosis. All dubious cases were omitted.

The *severity* of the thyrotoxicosis in the various cases is evident from Table 1.

In 20 per cent of the cases the past history of the patient furnished information about a previous appearance of a nontoxic goiter, usually nodular.

On the average, the anamnesis of the patients was short. Thus, a majority of them had had their symptoms for from six months to one year. Only 20 per cent of the patients had been ill for more than two years.

In 18 cases the thyrotoxicosis involved a recurrence after previous thyroidectomy. With regard to the symptomatology, the material differed in no way from the usual findings today, *i.e.*, loss in weight, circulatory phenomena, and enlargement of the thyroid were the predominant symptoms, whereas only a relatively few patients presented a pronounced exophthalmos.

TABLE 1. DEGREES OF THYROTOXICOSIS IN PATIENTS OF THE PRESENT STUDY

		Men	Women
Slight	(BMR < +40%)	4%	18%
Moderate	(BMR +40 to +60%)	29%	40%
Severe	(BMR > +60%)	67%	42%

METHODS

Dosage. Methylthiouracil (4-methyl-2-thio-uracil) was the preparation chosen for continuous treatment. The initial dosage was 250 milligrams three times daily, usually for one or two months. When the basal metabolic rate had fallen to a nearly normal level and the gain in weight had commenced, the dosage was lowered to 250 milligrams once a day, usually for from two to four months. The concluding dosage was quite low *i.e.*, 125 milligrams daily, decreasing to 125 milligrams thrice or twice a week. Gradually the patients were kept on this low dosage for a greater length of time, generally six months. It has been our impression that the patients get along better in this way, and that the frequency of relapse is less in the patients given the protracted treatment.

Thus the treatment generally extends over about one year. It is to be emphasized, however, that the small maintenance dosage was reached relatively rapidly and was then kept up for several months.

Duration of treatment. For the 48 patients whose treatment was considered to have been concluded, the duration of treatment varied from two to forty-two months, averaging 11.8 months (Fig. 1).

It is difficult to give any exact criteria for discontinuance of treatment. To a large extent, this is based on clinical judgment; but, as already mentioned, our experience indicates that it is preferable to extend the last phase of the treatment over a longer period than formerly. Thus we now hesitate to discontinue the treatment in less than nine months, extending it most often over one year. In practically all the cases the treatment was instituted in the hospital. The *duration of the hospitalization* varied con-

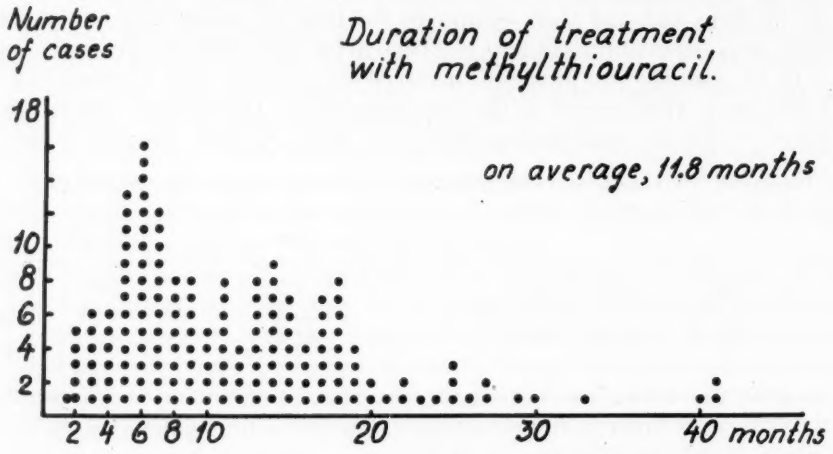

FIG. 1. Duration of treatment with methylthiouracil in the individual cases.

siderably, according to the character of the individual case. On an average, it was 27.0 days for men, and 33.4 days for women.

Those cases in which the hospitalization extended over months, were usually complicated, most often by cardiac disease but sometimes by accidental intercurrent conditions.

Gradually we have arrived at the view that the hospitalization may be cut shorter and that in a good many of the cases the entire treatment may be carried through ambulatorily, provided that the patients are under regular observation by a clinician fully acquainted with this form of treatment.

Upon discharge from the hospital, every patient is given written directions to the effect that he (or she) has to discontinue the treatment at once and get in touch with us personally or by telephone if he has 1) fever, 2) any skin eruption, 3) sore throat, or 4) if he feels indisposed in some other way.

The ambulatory control of the patient is somewhat individualized. At first the patients return once a month for examination and determination of the metabolism; later, at longer intervals.

We have omitted frequent control examination of the blood because this measure merely creates a false sense of security. If agranulocytosis develops, it generally sets in so suddenly that only in exceptional cases will the clinician be warned in time about its development.

RESULTS

Ability to work

When the patients are discharged from the hospital or transferred to ambulatory treatment, as a rule, they become able to work at once or very soon afterward. Nearly all the 48 patients in whom the treatment has been

TABLE 2. METHYLTHIOURACIL THERAPY DISCONTINUED IN 44 CASES BECAUSE OF:

Hypersensitiveness		25 cases
Compression of the trachea from transitory enlargement of the goiter		4 cases
Request by the patient	impatience	9 cases
	lack of cooperation	3 cases
	cosmetic reasons	1 case
Other causes	pregnancy	1 case
	continual relapses	1 case

concluded have been able, after their discharge, to resume work at once to a limited extent.

Thirty-five per cent of the total 244 patients have been able after their discharge to resume work at once to full and normal extent; 18 per cent have been capable of this after one month; 14.6 per cent after two months; and 20.5 per cent after three to six months. Two per cent of the patients claimed that they were unable to work on account of thyrotoxicosis, and 4 per cent did not work on account of their age.

As a matter of fact, we have gradually been more inclined to let the patient resume work sooner than formerly allowed. Once the more conspicuous thyrotoxic symptoms are abolished, the patient will usually be able to work under the continued treatment—provided, of course, that no complicating lesion is present.

For an evaluation of this form of treatment it is important to realize that continuous treatment does not mean protracted incapacity for work.

Cases in which treatment was discontinued

In 44 cases (18 per cent), the treatment with methylthiouracil had to be given up for various reasons (Table 2), chiefly because of hypersensitiveness to the drug.

Untoward effects

Among the total of 244 patients treated with methylthiouracil, untoward effects from the drug were observed in 29, or 12 per cent (Table 3). It is worth mentioning, however, that in 4 of these cases the treatment could be resumed in spite of the transitory allergic reactions. Subsequently, the assumed allergic nature of the reactions was verified in nearly every case by provocative dosage.

TABLE 3. UNTOWARD EFFECTS OBSERVED

	29 Cases
Drug fever	26 cases
Exanthem	15 cases
Muscle pain	3 cases
Conjunctivitis	5 cases
Agranulocytosis	0 cases
Leukopenia	4 cases
Headache	6 cases
Diarrhea	4 cases

The frequency of the reactions from hypersensitivity to the administered drug in these cases is recorded in Table 3. Sometimes the allergic reactions appeared as solitary phenomena, but most often they were combined in various ways.

Agranulocytosis, the most dreaded complication, was not seen in its typical form in any of our cases. On the other hand, in 4 cases a drug fever developed simultaneously with a state of *leukopenia;* the leukocyte count fell to 2,600–2,000, though with from 38 per cent to 56 per cent granulocytes. These manifestations cleared up spontaneously and promptly on discontinuance of the treatment, although 1 patient had to be given a blood transfusion and penicillin. These reactions were only slight, and 2 of the patients were able later to resume the treatment without any inconvenience.

The allergic reactions generally appeared after one to three weeks of treatment, that is, usually while the patients still stayed in the hospital. In 1 case, however, drug fever appeared after two years of treatment, so we may never feel quite secure in this respect.

Of the 25 patients in whom the methylthiouracil was discontinued on account of hypersensitiveness, 6 were at once transferred for thyroidectomy. In 18 of the remaining cases the patients were then given propylthiouracil, as this drug had become accessible to us. Fourteen of these 18 patients tolerated the new preparation well and were able to continue their treatment with it, whereas 4 patients were hypersensitive to the propyl compound, too. One of these 4 patients, however, was subsequently

able to resume the treatment with propylthiouracil, but the remaining 3 patients were transferred for operative treatment.

Cases in which treatment was continued

As mentioned before, the original material comprised 244 patients. Of this total, 9 patients died during the observation period from intercurrent causes that had nothing to do with their thyrotoxicosis. Three of them were still under treatment at the time of exitus. They died, respectively, of 1) uremia, 2) cancer of the uterus, and 3) abscess of the lung. None of these patients had manifest thyrotoxicosis at the time of death. As previously mentioned, therapy was discontinued in 44 other patients. Thus, the treatment with methylthiouracil was carried on in 191 cases.

In 22 patients this treatment is not yet concluded. This leaves 169 patients in whom the treatment had been either concluded or resumed on account of recurrence. It is this material that will be submitted to further analysis.

Among those cases in which the treatment was concluded at the time of the re-examination, there are 18 cases in which the treatment already had been discontinued once or oftener, and in which it had to be resumed subsequently because the patient had a relapse.

When the frequency of recurrence is being estimated for a study of thyrotoxicosis, distinction is usually made between true recurrence and cases in which the symptoms flare up again so early that they cannot be looked upon as a true recurrence but rather as a persistent thyrotoxicosis (or relapse). These early relapses are due to premature discontinuance of methylthiouracil therapy. Exacerbation of the symptoms within a period of six months is reckoned as *false recurrence* or *relapse.*

Under the heading of true recurrence, are entered those cases in which the conclusion of the treatment has been followed by a symptom-free period of at least six months before the reappearance of thyrotoxic symptoms. Of the 169 patients, at the time of re-examination, 7 had not yet passed the six-month limit after the discontinuance of the treatment.

Thus, there remain 144 patients who continued without any treatment for six months or more. In this group, the observation period after the discontinuance of treatment varied from six to sixty-three months, *averaging 32.9 months.*

State of health after conclusion of treatment

The state of health of the patients after the conclusion of the treatment is based on my own examination of *all* the patients. All the clinically noticeable symptoms of thyrotoxicosis were recorded, together with the body weight, and occasionally the rate of basal metabolism. At the same

time, of course, the patients were questioned thoroughly about possible complaints.

The rate of basal metabolism was determined in all patients showing any remaining symptoms or the least suspicion of recurrence. In the other patients a determination of the metabolism could not be carried through consistently. In our opinion, the clinical estimation of a given case may often be of more value than ambulatory determination of the metabolism, provided that the clinical examination is performed by one experienced in dealing with patients in this category.

The final outcome of the re-examination of the 144 patients in whom treatment with methylthiouracil has been discontinued for more than six months is recorded in Table 4, from which it will be noticed that 78 per cent were perfectly well.

TABLE 4. FINAL RESULT ON RE-EXAMINATION OF 144 PATIENTS

Complete recovery	78%
Improved, with certain persisting symptoms	18%
Recurrence	4%

The group "Improved, with some persistent symptoms" requires more detailed discussion. None of these patients showed manifest thyrotoxicosis with increased metabolism. The persistent symptoms here concerned are entered in Table 5.

TABLE 5. GROUP OF "IMPROVED, WITH SOME PERSISTING SYMPTOMS"

	(*27 Patients*)
Excessive perspiration	5 cases
Tremor of hands	2 cases
Retraction of eyelids	4 cases
Edema of eyelids	5 cases
Exophthalmos	10 cases
Auricular fibrillation	6 cases
Nervousness	21 cases
Fatigue	9 cases
Cardiac insufficiency	3 cases

The body weight was increased on an average by 7.3 Kg. as compared to the weight at the beginning of treatment. In 14 cases the weight had increased so markedly (up to 35 Kg.) that active reducing treatment was required.

Size of goiter

The behavior of the goiter has been followed with particular interest. Any exact estimation of the size of the goiter is difficult. We have chosen the method of describing carefully its form, size and consistency, on the basis of palpation. When, as here, the size of the goiter is estimated in every instance by the same examiner, the outcome ought to be reliable.

TABLE 6. SIZE OF GOITER BEFORE AND AFTER TREATMENT WITH METHYLTHIOURACIL

Goiter	Before treatment	On re-examination
Diffuse large	8.6%	2.6%
Diffuse moderate	47.5%	29.3%
Nodular	14.6%	15.1%
None	29.3%	53.0%

On the whole, the goiters were smaller at the time of re-examination than at the commencement of treatment; but, as was to be expected, no change took place in the size of the toxic *nodular* goiters. On the contrary, one goiter was added in this category by the re-examination (Table 6).

Exophthalmos

The estimation of the Basedowian eye symptoms is likewise based to a great extent on clinical judgment. Systematic registration of the degree of exophthalmos has been carried out only before and after the treatment and at the time of re-examination. The other Basedowian eye symptoms have not been taken into account (Table 7).

TABLE 7. EXOPHTHALMOS BEFORE TREATMENT AND ON RE-EXAMINATION

Exophthalmos	None	Moderate	Severe
Before treatment	69%	27%	4%
On re-examination	93%	5.4%	1.6%

In 4 patients, in whom prior to the treatment there had been a severe degree of exophthalmos, this symptom was completely absent on re-examination. On the other hand, 1 patient, in whom on admission there had been no exophthalmos, showed a very severe degree of exophthalmos, of a rather malignant progressive type, after the discontinuance of relatively brief treatment with methylthiouracil.

TABLE 8. INCIDENCE OF AURICULAR FIBRILLATION

Auricular fibrillation	Treated with digitalis	No digitalis
Present on admission	12 cases	2 cases
Appearing during treatment	2 cases	5 cases
Present on re-examination	6 cases	0 cases

Auricular fibrillation

Among the cardiac complications in thyrotoxicosis, particular interest was attached to the appearance of auricular fibrillation (Table 8). In 2 patients auricular fibrillation appeared after discontinuance of the methylthiouracil therapy. These patients were 57 and 72 years old, respectively.

Significance of preceding iodine therapy

Twenty-five patients had been treated at home with iodine prior to the institution of the methylthiouracil treatment. In these instances, iodine had been given for an average of six months. The duration of treatment for the patients who were treated with iodine beforehand was 17.1 months on the average, against 11.7 months for the patients treated exclusively with methylthiouracil. As to the late results and frequency of recurrence, there appears to be no difference between the two groups.

Thus the preceding iodine therapy prolonged the necessary period of treatment with methylthiouracil—probably because treatment with iodine produces involution of the thyroid with deposition of the hormone in the gland, thus delaying the subsequent effect of the antithyroid substance.

Frequency of recurrence

Among the 144 patients with an observation period of over six months (averaging nearly three years) a true recurrence appeared once in 18 patients and twice in 3 patients (Table 9).

After a perfectly symptom-free period of fairly long duration, thyrotoxic symptoms began to appear again in these 21 patients, on which account

TABLE 9. FREQUENCY OF RECURRENCE IN 144 PATIENTS

False recurrence (relapse)		14.5%
True recurrence	once	12.5%
	twice	2.1%
	thrice	0
Total		16.6%

the treatment was instituted anew. The symptom-free period lasted between six and forty-five months (Fig. 2).

These figures do not give the actual frequency of recurrence, however, as the last account does not include the 18 patients in whom the treatment

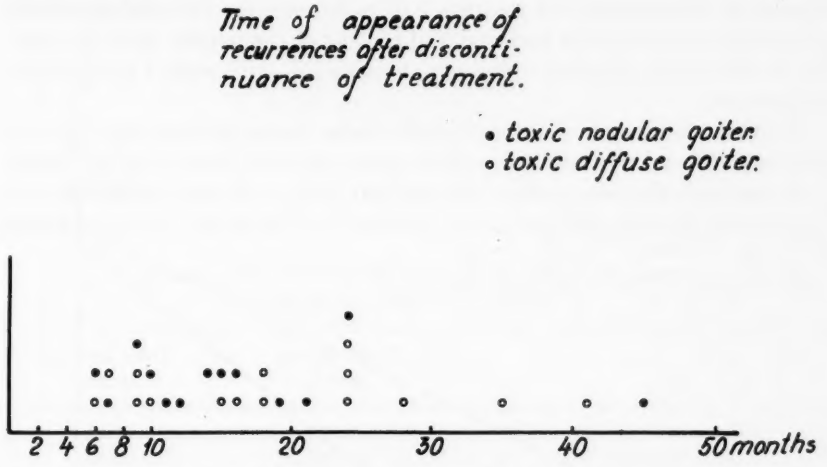
Time of appearance of recurrences after discontinuance of treatment.
• toxic nodular goiter
∘ toxic diffuse goiter
2 4 6 8 10 20 30 40 50 months

FIG. 2. Time of the appearance of recurrence after discontinuance of methylthiouracil therapy.

was repeated just on account of recurrence and not yet concluded at the time of the re-examination. On reckoning these cases together with the others we find the figures for recurrence to be somewhat higher (Table 10). The figures given in Table 10 are maximum values, some of the cases being rather dubious, and the recurrence in most of the remaining cases being of a rather mild character. We found it preferable in these instances to repeat the treatment at once rather than to wait for the symptoms to become more pronounced.

On the average, the recurrences were easy to deal with and thus the

TABLE 10. FREQUENCY OF RECURRENCE IN 169 PATIENTS IN WHOM THE TREATMENT HAS BEEN CONCLUDED OR RESUMED

False recurrence (relapse)		18.3%
True recurrence	once	16.6%
	twice	3.5%
	thrice	0.6%
Total		25.5%

patients could soon be given a small maintenance dose. The final results in these cases have been as good as those obtained in the other group.

This frequency of recurrence was considerably higher than originally expected. Hence it may be of special interest here to observe which patients were particularly liable to a recurrence of their lesion. There was no fundamental difference in the frequency of recurrence for the two sexes; nor, according to the present material, did the age of the patient and the severity of the disease appear to play any decisive rôle with regard to a possible recurrence.

On the other hand, if we divide the entire material into cases of *toxic diffuse goiter* and cases of *toxic nodular goiter*, we meet with a distinct difference between the two groups, the nodular goiters showing recurrence far more often than the diffuse goiters (Table 11). The group of toxic nodular

TABLE 11. FREQUENCY OF RECURRENCE IN TOXIC DIFFUSE AND IN TOXIC NODULAR GOITER

	Toxic diffuse goiter	Toxic nodular goiter
False recurrence (relapse)	12.8%	25%
True recurrence	11.9%	30%

goiters comprises solitary as well as multiple adenomas. Naturally, the distinction between nodular and diffuse goiters will be based merely on palpatory findings, and this means a rather rough estimate.

Not only is the frequency of recurrence greater in the patients with toxic nodular goiter but the *therapeutic results are also poor* for this group, as is evident from Table 12.

The *duration of treatment* was longer for the patients with toxic nodular goiter and the effect of methylthiouracil was delayed more often in these patients, as is evident from Figure 3.

Furthermore, the gain in weight was less pronounced for the patients with toxic nodular goiter.

TABLE 12. LATE RESULTS IN TOXIC DIFFUSE AND IN TOXIC NODULAR GOITER

	Toxic diffuse goiter	Toxic nodular goiter
Complete recovery	79.5%	50%
Improvement	18.0%	40%
Recurrence	2.5%	10%

This difference in the results of methylthiouracil therapy in toxic nodular goiter and in toxic diffuse goiter forms a striking contrast to the results obtained with operative treatment, the final results of which appear to be much alike for the two groups. On the other hand, the frequency of recurrence in toxic nodular goiter is minimal compared to that in toxic diffuse goiter.

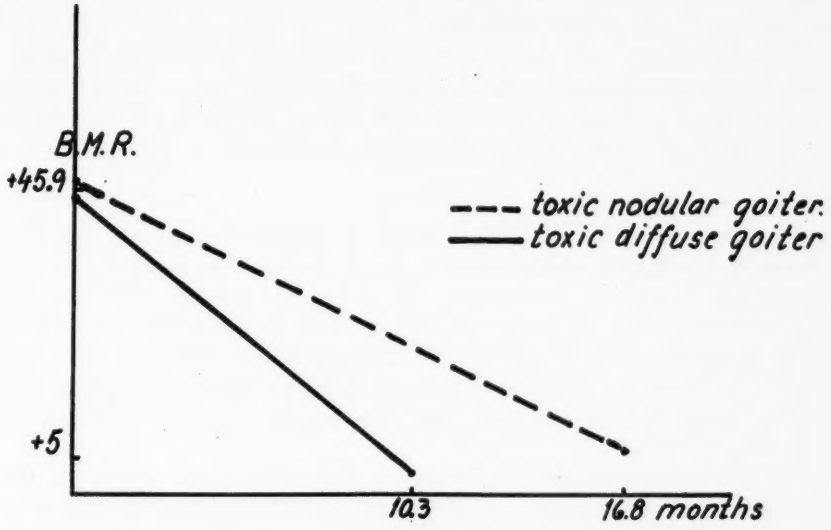

FIG. 3. Relation between the duration of treatment and return of the basal metabolic rate to normal level in cases of toxic nodular and of toxic diffuse goiter.

DISCUSSION

As mentioned in the introduction, continuous treatment with methylthiouracil was planned originally in our department as an experiment in which we would try to carry through this form of therapy in all the thyrotoxic patients referred to our clinic.

In the course of this study some of the patients gave up, or methylthiouracil therapy was given up in favor of other treatment (18 per cent). Still the experiment shows that, apart from these 18 per cent of the cases, it has been practicable to treat all cases of thyrotoxicosis after this therapeutic principle. On the other hand, an analysis of the late results, especially the frequency of recurrence, has shown that such consistent methylthiouracil therapy is not the ideal treatment in all cases of thyrotoxicosis.

The therapeutic results obtained in the group of patients with toxic

nodular goiter is considerably poorer than in the group with toxic diffuse goiter, and the frequency of recurrence is so high that in the future we shall primarily advise such patients to submit to operative treatment.

In a certain number of cases, moreover, there will be some entirely social indications for rapid cure by operative treatment. This can be taken for granted, and we are inclined to think that in the future this factor will be taken into consideration to a greater degree than before, when the method of treatment is to be decided upon.

The advantages from the conservative treatment are so great, however, and the results obtained in this way are still so good that we shall keep on employing the continuous treatment with methylthiouracil, although keeping in mind the significant reservations just mentioned.

SUMMARY

In order to try out the effectiveness of methylthiouracil in the conservative treatment of thyrotoxicosis in the Medical Department B of the Bispebjerg Hospital, Copenhagen, all of the 244 patients admitted for thyrotoxicosis since February 1944 have been given methylthiouracil as the only therapy. The dosage has been 250 milligrams three times daily for one to two months, then 250 milligrams daily, and finally 125 milligrams twice a week for a maintenance dose. The average duration of treatment has been 11.8 months.

In 44 patients this form of treatment had to be given up for various reasons. In 25 of these cases the treatment was discontinued on account of hypersensitiveness, but no instances of agranulocytosis occurred. Eighteen of these 25 patients were able to continue treatment with propylthiouracil.

In 144 cases the treatment was concluded in more than six months. The observation period varied from six to sixty months. On follow-up examination, 78 per cent of these patients were found to be perfectly well, 18 per cent were improved (with some persistent symptoms), and 4 per cent showed a recurrence of the disease. On re-examination, the goiter was found on the whole, to be smaller than at the beginning of treatment. Exophthalmos was found in 7 per cent of patients at the time of re-examination, compared to 31 per cent before treatment. Twenty-one patients had auricular fibrillation prior to treatment, compared with 6 at the time of re-examination.

In the total of 169 cases which form the basis of this study, there was a false recurrence (relapse) in 18.3 per cent and a true recurrence in 25.5 per cent. On the whole, the persistent and recurrent cases have been mild and soon responsive to a small maintenance dose; the final results, in cases in which treatment has been concluded, have been just as good as in non-recurrent cases.

The high frequency of recurrence is found in part to be due to toxic nodular goiter. When these cases are omitted from the material, the frequency of true recurrence in toxic diffuse goiter is 11.9 per cent.

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POLYOSTOTIC FIBROUS DYSPLASIA ASSOCIATED WITH HYPERTHYROIDISM

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POLYOSTOTIC fibrous dysplasia, when associated with endocrinopathies and pigmentation of the skin, has been termed "Albright's syndrome" following the report in 1937 by Albright *et al.* (1). The most common endocrine dysfunction is precocious puberty in females. Less frequent but thought to be significantly related is the presence of hyperthyroidism.

This report reviews all cases in the literature and presents 2 additional cases in which there was coexistence of hyperthyroidism and fibrous dysplasia. For detailed radiologic, pathologic and clinical features of this syndrome, the reader is referred to the recent excellent reviews by Furst and Shapiro (2), Albright (3, 4), Dockerty *et al.* (5), Lichtenstein and Jaffe (6) and Gorham *et al.* (7).

In Table 1 only those cases in which the diagnosis of hyperthyroidism is unequivocal are included.

The cases of Horowitz and Cantarow (12), Caldwell and Broderick (13), Dockerty *et al.* (5), Gaupp (14), Summerfeldt and Brown (15) and Thannhauser (16) (which are not included in Table 1) contain features such as moderately elevated basal metabolism and exophthalmos, which suggest that mild hyperthyroidism may have been present. In none of these cases, however, were the clinical signs or laboratory tests conclusively diagnostic of hyperthyroidism.

The following 2 cases, which will be described in detail, manifested the typical bony changes and pigmentation of Albright's syndrome and evidence of hyperthyroidism. In neither instance was a history of precocious puberty obtained.

Case 1. E. C., Hosp. No. 452–711

This 54-year-old Caucasian female was first seen by the authors in April 1949, when she was referred for admission to the hospital by the outpatient department for study of her thyroid function. At the age of 8 the patient had fallen, fracturing her right hip.

In 1935, when she was forty years of age, the patient first appeared in the outpatient department complaining of a 20-pound weight loss in spite of good appetite, weakness, palpitation, shortness of breath, nervousness, tremor and variation in the size of her thyroid gland for the preceding three years. Examination revealed tachycardia, slight

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TABLE 1

Case	Sex	Age (yrs.)	Bony involvement	Pigmentation	Precocious puberty	Clinical hyperthyroidism	BMR, %	Cholesterol, mg. %	Exophthalmos	Thyroid pathology	Remarks
1. McCune* & Bruch (8)	F	11	Widespread	Present	Present	Marked	+33 to +120	132, 145	Present	"Adenomatous hyperplasia"	Autopsy: basophilic adenomata of pituitary
2. Musser & Barnwell (10)	M	11	Moderate	"	Absent	Marked	+40 to +60	78	?	"Diffuse colloid goiter"	Incomplete autopsy
3. Peck & Sage (11)	M	22	Widespread	"	Absent	Mild	+22 to +45	150	Present	Goiter	Diabetes mellitus present
4. Albright & Reifenstein (4)	F	13	Moderate	"	Present	Moderate	+40	127	Absent	"Colloid goiter"	
5. Yettra & Starr (Case 1)	F	54	Widespread	"	Absent	Moderate	+41 to +51	135, 151	Present	"Adenomatous hyperplasia"	
6. Yettra & Starr (Case 2)	F	50	Widespread	"	Absent	Mild	+42	—	Absent	Goiter	Blood iodine, 11.6 micrograms per cent

*This case with autopsy findings reported by Sternberg and Joseph (9).

exophthalmos, and tremor. Blood and urine were normal except for slight hypochromic anemia; Wassermann and Kahn tests gave negative reactions. Serum cholesterol values were 135 mg. per cent and 151 mg. per cent and the B.M.R.'s varied from +41 to +51 per cent. After preparation with Lugol's solution, a two-stage subtotal thyroidectomy

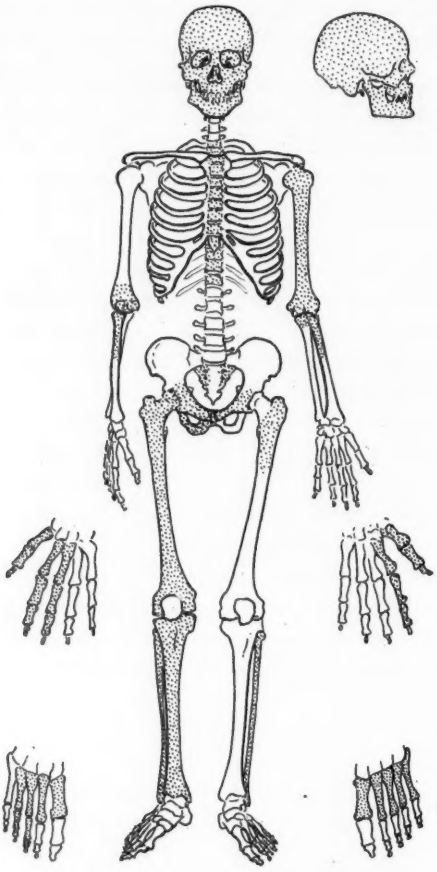

FIGURE 1.

was performed. The pathologic report was "adenomatous hyperplasia." The patient had a smooth postoperative course and was discharged much improved.

She was readmitted in December 1938 with a fracture of the right hip, which had occurred during a fall. X-ray studies revealed that the fracture was through a large cystic area in the greater trochanter of the right femur. An x-ray survey revealed numerous similar lesions which are diagrammed in Figure 1. Blood chemical studies at this time (December 1938) showed: calcium, 9.3 mg. per cent; phosphorus, 3.6 mg. per cent; alkaline phosphatase, 4.4 Bodansky units; and cholesterol, 185 mg. per cent.

No union had occurred by February 1939, so an open reduction was performed. The surgeon stated: "The marrow cavity contained numerous cystic areas filled with orange-yellow material." At this time the cysts were curretted, and fibrous tissue was removed. In spite of surgery, there was still no union and in September 1940 a vitallium plate and bone graft were inserted. Union ultimately took place.

In January 1949 the patient was again seen in the clinic, complaining of increasing nervousness. No other symptoms of hyperthyroidism were present. Examination revealed a few, small, firm nodules beneath the thyroidectomy scar. Blood and urine were

FIG. 2. A reproduction of a snapshot taken when the patient (Case 1) was 30 years old. The pigmentation on the left arm has faded considerably in the interim.

normal. Protein-bound blood iodine determinations varied from 6.0 to 9.3 micrograms per cent (normal 5–7).

The menarche occurred at the age of 13 and subsequent menstrual periods were normal. She had one pregnancy with delivery of a normal full-term infant. Her son at present is in the Army and has no evidence of bony disease.

Physical examination on this last admission (April 1949) revealed a thin, alert woman in no distress. The head appeared larger than normal due to prominence in the frontal and occipital regions. There was a faint patchy area of pigmentation on the left upper arm and over the left scapula, with an irregular outline of the "coast-of-Maine" type as described by Albright (Fig. 2). Exophthalmos was not present. The ramus of the left mandible was enlarged to several times its normal size by a fusiform bony mass. A collar thyroidectomy scar was present and beneath it two small, firm, fixed nodules could be felt (Fig. 3). Heart and lungs were normal. There was a marked kyphoscoliosis. In the abdomen both kidneys could be easily palpated, but they were of normal size. The liver edge was felt two finger-breadths beneath the right costal margin. There was shortening and external rotation of the right leg. Blood chemical studies (April 1949) showed: calcium, 8.4 and 8.2 mg. per cent; phosphorus, 3.4 and 2.6 mg. per cent; alkaline phosphatase, 7.6 and 8.8 Bodansky units.

FIG. 3. (Case 1). The fusiform enlargement of the mandible is well shown.

After administration of 100 microcuries of I^{131}, a pickup of 22 per cent at twenty-four hours was found, indicating normal thyroid function at this time. Urinary calcium excretion (after a 3-day calcium diet providing 100 mg. of calcium daily) was 890 mg. in twenty-four hours, indicating a marked negative calcium balance.

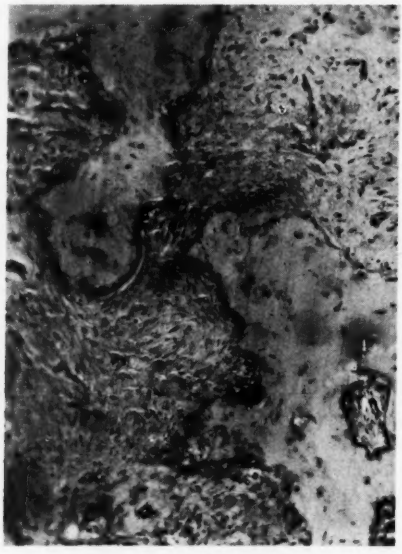

FIG. 4. Histologic section of mandibular biopsy specimen (Case 1) ×120.

A biopsy specimen of the mandibular cyst revealed the typical changes of fibrous dysplasia of bone (Fig. 4).

Case 2. L. B., Hosp. No. 458-149

A 50-year-old Caucasian, married, deaf-mute housewife was admitted to the hospital in August, 1949. She wrote that at the age of 1 she fell down some stairs, injuring her head. This accident left her totally deaf. She had not spoken since that time. At the age of 3 her left forearm was broken. The patient did not remember how she incurred this

FIG. 5. (Case 2). Note the pigmentation on the lower lip.

fracture. She had marked "bowlegs" as a child, and when 4 years old a doctor attempted to correct this deformity by "breaking" both legs and resetting them. She fractured her left tibia at the age of 16, following minimal trauma; and her left femur at 21, by falling. She had had pigmentation on her face and back since birth and on her lips since the age of 21.

The menarche took place at 13 and the menstrual history was normal except for menorrhagia from 1944 to 1946. The menopause occurred in 1947. In 1942 she had a normal pregnancy and was delivered in this hospital by cesarian section, because of marked deformity of the pelvis. The child is normal in every way.

She noted slight heat intolerance, but no tremor. She had 2 to 3 normal bowel movements daily. She was slightly "nervous" and suffered from occasional frontal headaches. System review showed nothing abnormal. Locomotion was limited because of deformity of her legs, but she was able to get around on crutches.

She was first seen in this hospital in 1937, at which time a simple mastectomy was performed for "intracystic papillary adenoma." At this time her basal metabolic rate was +42 per cent, serum calcium 10 mg. per cent, phosphorus 2.39 mg. per cent, and

alkaline phosphatase 8.4 Bodansky units. She was next seen in 1942 when she had her cesarian section. In 1944 she fell while holding her baby and suffered a dislocation of the right hip with a pathologic fracture of the femur. On this admission, serum calcium was 9.7 mg. per cent, phosphorus 2.3 mg. per cent, and alkaline phosphatase 6.5 Bodansky units.

Physical examination revealed an alert, intelligent, slightly obese woman with prominence of the head in the frontal region. Her temperature was 99.6, pulse 80, respiration 22, and blood pressure 180/100.

There was a rounded, fawn-colored area of pigmentation over the left side of the face,

FIG. 6. (Case 2). The area of pigmentation over the sacrum. Note the irregular ("coast-of-Maine") outline.

with a smooth border. On the lower lip and on the buccal mucosa were several small irregular areas of dark pigmentation (Fig. 5) and over the sacrum was a 3 in. × 5 in. area of dark pigmentation with an irregular border (Fig. 6). There was a sensation of fullness over the thyroid and a 2 cm. nodule was felt in the left lobe. No bruit was heard. The back was involved in marked kyphoscoliosis. The lungs were normal except for a few medium moist rales at the bases. The heart was not enlarged; rhythm was regular; and a Grade II blowing systolic murmur was heard at the apex. The tip of the spleen was felt two finger-breadths below the left costal margin; it was firm. There was marked shortening and deformity of both lower extremities, with nodular enlargement of the cortices of the bones. Similar changes were present in the left radius and ulna. An x-ray survey revealed widespread areas of skeletal involvement by fibrous dysplasia, as diagrammed in Figure 7.

The urine was normal and the Wassermann and Kahn tests gave negative reactions. Blood chemical studies showed: calcium, 10.6 mg. per cent; phosphorus, 3.0 mg. per cent;

alkaline phosphatase, 9.1 Bodansky units; and protein-bound iodine, 11.6 micrograms per cent (normal 5–7).

An electroencephalogram showed no abnormality. Mandibular biopsy findings indicated "fibrous dysplasia of bone" (Fig. 8). Further studies were contemplated but the patient was unable to remain in the hospital for their completion.

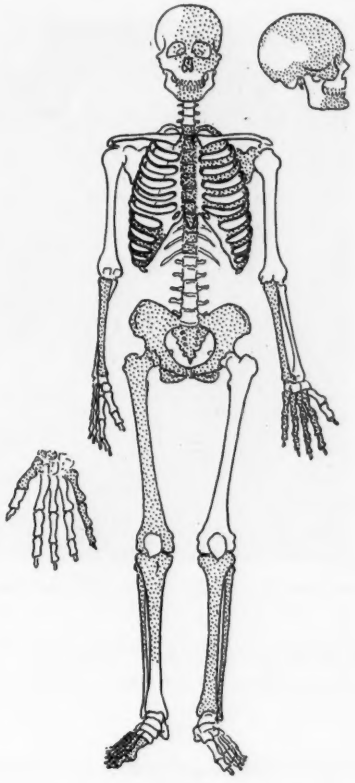

FIGURE 7.

COMMENT

Polyostotic fibrous dysplasia is a nonfamilial and nonhereditary condition in which lesions of bone, pigmentation of the skin and certain endocrine disorders may coexist. The paucity of reports of complete postmortem examinations makes it unwise at the present time to speculate on the pathogenesis of this syndrome. The endocrine dysfunction is not incompatible with normal parturition.

In about 5 per cent of cases, hyperthyroidism is present. It seems likely

that the relation between these two diseases is more than coincidental. An elaboration of the relationship between the bony and endocrine aspects of this entity may lead to further understanding of the etiology of hyperthyroidism.

When mandibular lesions are present, as they often are (17), pathologic

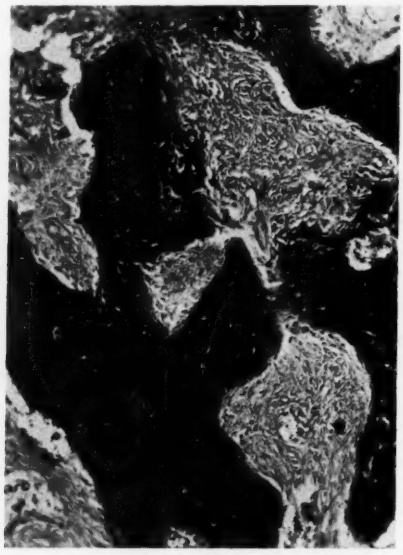

FIG. 8. Histologic section of mandibular biopsy specimen (Case 2) ×120.

confirmation of the diagnosis is easily obtained by intra-oral mandibular biopsy, with the specimen taken under local anesthesia.

SUMMARY

Two cases of polyostotic fibrous dysplasia with coexisting hyperthyroidism are presented, and 4 similar cases from the literature are reviewed.

Acknowledgment

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ADDISON'S DISEASE IN THE NEGRO

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INTRODUCTION

ADDISON'S disease apparently occurs infrequently in the Negro. Since Scheult's (1) original description in 1907, only 27 case reports have appeared in the literature. The clinical diagnosis may be difficult, as pigmentary changes are often inconspicuous. Furthermore, there is question as to whether the incidence of this disease may actually be less in the Negro than in the Caucasian (2). In this report, are presented 4 cases of Addison's disease in the Negro in which the diagnosis was made clinically. Striking changes in pigmentation which occurred in 2 patients made them of unusual interest. One patient demonstrated the effect of slowly progressive adrenal insufficiency on pre-existing hypertension. The latter patient tolerated well a surgical procedure of moderate severity with the aid of Lipo-adrenal Cortex Extract and cortisone.

CASE REPORTS

Case 1. B. H., a 39-year-old Negress, was admitted for the first time to Barnes Hospital on August 8, 1948, with the complaints of weakness, a 27-pound weight loss, and increased pigmentation. These symptoms had begun three months previously when the patient had a sore throat, and became critically ill with fever, anorexia, nausea and vomiting. During the second week of this illness she noted increased pigmentation of her face, hands, and palms. The febrile illness was treated with penicillin and sulfadiazine. Six weeks prior to her entry to Barnes Hospital, she was given blood transfusions and vitamins by her physician and was told that her blood pressure was low.

On admission, the patient was thin and weak. The blood pressure (supine) was 96/60 mm. of Hg. Her skin was smooth and showed generalized dark brown pigmentation. The pressure areas over the elbows, knuckles, and sacrum were dull, and dark grey to black in color. The linear markings of the palms were broad, dark brown streaks (Fig. 1). The gums, tongue, and buccal mucosa were also deeply pigmented. The remainder of the physical examination showed nothing abnormal.

Laboratory studies revealed normal results for blood counts, Kahn test, urinalysis, and stool examination. The fasting blood sugar was 79 mg. per cent. The blood electrolytes, all expressed as milliequivalents per liter, were as follows: carbon dioxide combining power, 24; serum chloride, 99; and serum sodium, 135.9. The basal metabolic rate was minus 15 per cent. No fall in eosinophils occurred four hours after 25 mg. of ACTH (3). Part I and Part II of the Robinson-Power-Kepler water test (4) gave positive results. The urinary 17-ketosteroid excretion was 2.2 mg. per twenty-four hours. A first-

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strength tuberculin skin test showed a positive reaction. The roentgenogram of the chest showed bilateral apical tuberculosis; however, sputum smears and guinea pig inoculation with the gastric contents did not reveal any tubercle bacilli. An intra-abdominal calcification lying above the upper pole of the left kidney could be seen on an x-ray film of the abdomen.

The patient was given 5 mg. of desoxycorticosterone acetate and 9 Gm. of sodium chloride daily. She gained weight and lost her symptoms of anorexia, vomiting and weakness. Her blood pressure rose only slightly. She was given 0.5 Gm. of streptomycin daily for thirty-one days, with the hope of altering the tuberculosis. On September 14,

FIG. 1. Case 1.

1948, six pellets, each containing 75 mg. of desoxycorticosterone acetate were implanted subcutaneously. Following discharge from the hospital on September 25, 1948, the patient did well. She used no added salt. Her weight and blood pressure rose gradually. After eighteen months, she was readmitted for another implantation of pellets.

Physical examination made on March 3, 1950, showed a thin, darkly pigmented woman. She thought her pigmentation had decreased. Her lungs now had fine moist rales and dullness at both apices. Blood pressure (supine) was 125/80 mm. of Hg. Four pellets (75 mg. each) of desoxycorticosterone acetate were implanted by trocar. The patient was discharged to be cared for in a tuberculosis sanatorium.

Case 2. B. M., a 42-year-old Negress was first admitted to Barnes Hospital on May 25, 1931, with a painful mass in the region of the lower thoracic vertebrae. A diagnosis of tuberculosis of the vertebrae and surrounding soft tissue was made. At that time, the body skin was light tan in color (Fig. 2). The blood pressure was 120/75 mm. of Hg. The urine was normal.

The patient was readmitted several times because of extension of the tuberculous process to the lumbar vertebrae and to the soft tissues of the right hip.

In July 1949, the patient reported to the Washington University Clinics and complained of increased generalized pigmentation of eighteen months' duration, pain in the back and right hip of ten months' duration, and swelling of the feet. Her blood pressure, during the months from July 1949 to January 1950, varied from 156/92 mm. of Hg to 200/125 mm. of Hg. In October 1949, the patient was studied in the endocrine clinic

FIG. 2. Case 2, May 1931.

because the increased pigmentation suggested Addison's disease. A Thorn test made at that time, with 0.2 mg. of epinephrine hydrochloride, showed a 50 per cent fall in eosinophils after four hours. The basal metabolic rate was minus 5 per cent. In September 1949, following the ingestion of 75 Gm. of glucose, the blood sugar values were as follows: fasting, 71 mg. per cent; first hour, 90 mg. per cent; second hour, 81 mg. per cent; third hour, 54 mg. per cent; fourth hour, 65 mg. per cent; and fifth hour, 74 mg. per cent. On January 10, 1950, her blood pressure was 150/100 mm. of Hg.

The patient returned to the clinic on March 12, 1950. During the two months since she was last seen, nausea, vomiting and anorexia had developed, with a weight loss of 25 to 30 pounds. Weakness had become so marked that the patient was unable to sit up in bed for more than thirty minutes a day. During the previous year, her usually regular menstrual periods had become irregular, and frequently she skipped a month. She was readmitted to Barnes Hospital on March 12, 1950, because of these complaints.

On physical examination, the blood pressure was 110/70 mm. of Hg, reclining; and 90/60 mm. of Hg, standing. The skin over the entire body was dark brown (Fig. 3). The creases in the palms were likewise deeply pigmented (Fig. 4). The soft palate, buccal

mucosa, and the under surface of the tongue showed bluish-black areas. A moderate dorsal kyphosis was present and motion of the right hip was limited. The laboratory findings revealed the following values: red blood count 3,000,000; hemoglobin 11.5 Gm.; white blood count 6,000; the differential white blood count showed 61 per cent lymphocytes; and the sedimentation rate (Wintrobe) was 35 mm. per hour. A Thorn test, using 0.2 mg. of epinephrine hydrochloride, showed a rise from 40 to 120 eosinophils per cu. mm. after four hours. Eosinophil counts following administration of 25 mg. of ACTH showed a rise from 62 to 91 eosinophils per cu. mm. Both parts of a Robinson-Power-Kepler water test (4) gave positive results. Urinary gonadotropin determinations[1] during two successive 24-hour periods showed 31 and 34 mouse-uterus units (normal values: 15–

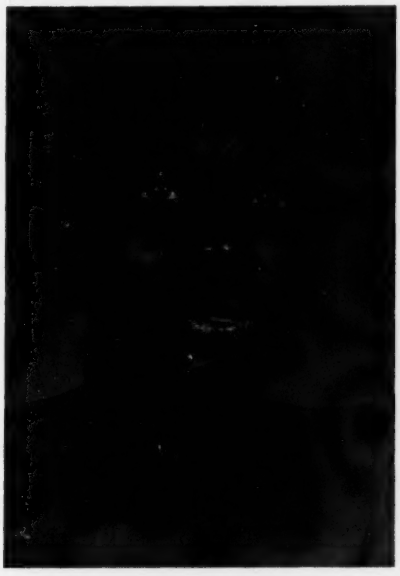

FIG. 3. Case 2, March 1950.

20 m.u.u.). The basal metabolic rate was minus 35 per cent. The blood cholesterol level was 162 mg. per cent. A Congo red test for amyloid disease gave negative results. An oral glucose tolerance test again showed a flat curve. The urinary corticosteroids[2] were 0.86 mg. and 0.32 mg. during successive 24-hour periods (normal value: 0.5 mg. to 1.5 mg. per twenty-four hours). Roentgenograms of the right hip at this time showed destruction of the greater trochanter with sequestration. Because of pain in this hip and disability, removal of the greater trochanter and adjacent soft tissue was thought necessary.

Two weeks prior to this operation the daily administration of streptomycin (1 Gm.)

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[1] Gonadotropin was determined by the method of A. Gorbman, described in *Endocrinology* **37**: 177–190, 1945.

[2] Corticosteroids were determined by the method described by W. H. Daughaday, H. Jaffe and R. H. Williams in *J. Clin. Endocrinol.* **8**: 166–174, 1948.

and para-amino salicylic acid (10 Gm.) was started. The latter had to be discontinued because of the vomiting it provoked. She was given desoxycorticosterone acetate, 7.5 mg. daily by intramuscular injection, and a diet containing 6 Gm. of sodium chloride daily. This therapy maintained her blood pressure at a normal level (average 120/85 mm. of Hg), and her weight remained stable. Three days before operation Lipo-adrenal Cortex Extract was started (1 cc. three times daily intramuscularly). The blood pressure did not rise but the fasting blood sugar increased. In addition to the above medications, on the day of the operation she received 25 mg. of cortisone intramuscularly every six hours for a total of three doses. Her total salt intake that day was about 12 grams. For the only

FIG. 4. Case 2, March 1950.

time during her hospitalization, her blood pressure rose to hypertensive levels (150/100 mm. of Hg). The following day the blood pressure was normal. The operation was performed under novocaine anesthesia. For the first three postoperative days, the patient continued to receive Lipo-adrenal Cortex Extract, in addition to desoxycorticosterone acetate. The latter was continued until pellets were implanted. The patient did well until the tenth postoperative day, when pneumonia of the entire right lung developed; this completely resolved in one week. Three pellets (75 mg. each) of desoxycorticosterone acetate were implanted subcutaneously and the patient was discharged to a tuberculosis sanatorium.

Case 3. H. G., a Negro male, aged 50, was admitted to Barnes Hospital on October 16, 1930. The patient had been ill for two months with vomiting, cough, left flank pain, and weight loss. On physical examination, there was brown pigmentation of the hard palate but no other pigmentary changes were detected. The heart was small and the sounds were distant. When the patient was supine, the blood pressure was 98/64 mm. of Hg; but

after a few minutes in the erect position, the pressure dropped to 60/0 mm. of Hg. The clinical diagnosis was Addison's disease. During his hospital stay, the patient had a constant fever until his death. Autopsy showed caseous tuberculosis of the lungs, liver, abdominal lymph nodes and the adrenals.

Case 4. D. T., a Negro male, aged 40, was admitted to Barnes Hospital on July 14, 1936. His complaints at the time of entry were nausea, vomiting, abdominal pain, and weight loss. The patient had had an elevated temperature for several weeks prior to admission. On examination, there was an unusual dark pigmentation of the hard palate, and a bluish-gray line along the lower gingival margin. The blood pressure was 104/52 mm. of Hg. No other significant abnormalities were detected. The patient continued to vomit, and remained hypotensive and febrile until his death. The clinical diagnosis of Addison's disease was made. Autopsy revealed a tuberculous pneumonia, and caseous destruction of the adrenals, prostate, seminal vesicles and epididymides.

DISCUSSION

Despite the scarcity of reported cases of Addison's disease in Negroes, Conn and others (2) have postulated that probably many occur. They are not recorded, however, for various reasons such as insufficient interest in the cases or actual failure to recognize them. Conn, on the basis of a series of cases seen at the University of Michigan Hospital, demonstrated statistically, that the disease is more common in Negroes than in Caucasians. The experience of others, however, does not bear this out. Thorn (5) has seen cases of Addison's disease in the Negro but he has no case report of any in his current detailed studies of over 200 patients with adrenal insufficiency. Blache (6) reports that of 2,800 autopsies performed between 1935 and 1949, at the Homer G. Phillips Hospital (St. Louis City Hospital for Negroes), only 1 probable case of Addison's disease has been found. However, at the Barnes Hospital, out of a total of 66 cases of Addison's disease, 4 were in Negro patients, a ratio of 16 to 1. The ratio of hospital admissions of white patients to Negro patients is 13 to 1. From the data of Conn and of the Barnes Hospital, it is apparent that Addison's disease occurs more frequently in the Negro than has been recognized in the past. Because of these conflicting observations, it is impossible for us to draw any conclusions as to the actual incidence of Addison's disease in Negroes.

Excluding Lowrie's patient (7), who had a pulmonary lesion not diagnosed in his report, tuberculosis has been the etiologic agent in all but 1 case of Addison's disease reported in Negroes. This 1 case described by Conn (2) was due to amyloid infiltration of the adrenal cortex. This is in direct contrast to the situation in white patients, in whom only about 50 per cent of cases of Addison's disease are due to tuberculous destruction of the adrenal; and in whom most of the remaining cases are due to primary atrophy of the cortex (8). On the basis of the previously reported cases of Addison's disease in the Negro, and the 4 cases herein reported, it would seem possible that the Negro has a resistance to agents causing atrophy,

and his poor resistance to tuberculosis probably makes him more susceptible to tuberculous destruction of the adrenal gland.

The greatest difficulty in making this diagnosis in the Negro is the inability of the physician to recognize abnormal pigmentation. In many Negroes pigmentation of the mucous membranes, of palmar creases, and of scars may occur normally. Two of the patients described here initially had light tan skins which became dark brown-black in color with the development of adrenal insufficiency. The pigmentary changes obviously are masked in the dark Negro. Soffer (9) remarks that in the absence of increased pigmentation, the diagnosis of Addison's disease is hazardous.

Other conditions may give rise to increased tanning of the skin, one of the most confusing being acanthosis nigricans. Like Addison's disease, this condition will cause cutaneous as well as oral pigmentation. If the acanthosis is associated with carcinoma, as it is in 50 per cent of cases (10), it may further mimic Addison's disease by being associated with weakness, weight loss, nausea and vomiting. Even the 17-ketosteroid excretion may be low because of the general debilitation. Papules and verrucose skin lesions which occur in this condition aid in differentiation from Addison's disease.

In 1 of the cases reported here (Case 2) increased pigmentation antedated all other signs and symptoms of Addison's disease by nearly two years. This dissociation of onset of pigmentation and symptoms of adrenal insufficiency has been noted before (9).

The diagnosis of Addison's disease could not be made with certainty in Case 2 when the patient was first seen in July 1949, because of the definite hypertension and also because the Thorn test showed a prompt 50 per cent fall in eosinophils after four hours. As the disease progressed, the blood pressure fell to normal or below and the response to the Thorn test became abnormal. Only during the time the patient received cortisone did she again have hypertension. Perera (11) has also observed that cortisone may raise the blood pressure in patients with adrenal insufficiency. That the destruction of the adrenal cortex was progressive is evidenced by the development of hypotension and an abnormal response to the Thorn test.

SUMMARY

Four cases of Addison's disease in Negroes, diagnosed during life, are presented. Tuberculous destruction of the adrenal cortices was demonstrated at autopsy in 2 of the cases. The other 2 cases presumably also were due to tuberculosis because other lesions of tuberculosis were present. Marked increase in the pigmentation in 2 of the cases suggested the diagnosis.

Acknowledgments

We wish to express our appreciation to Dr. C. M. MacBryde and Dr. W. H. Daughaday, of the Metabolism Laboratory of Barnes Hospital, for determination of the 17-ketosteroids and corticosteroids. We also wish to thank Dr. D. T. Magallon and Dr. W. H. Masters, of the Department of Obstetrics and Gynecology at the Washington University School of Medicine, for the determinations of urinary gonadotropins.

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MENARCHE AND PREGNANCY AFTER REMOVAL OF AN ADRENOCORTICAL ADENOMA*

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From the Escola Paulista de Medicina, Sao Paulo, Brazil

THE clinical and laboratory manifestations of hyperfunctioning adrenocortical tumors are variable, depending upon the character of the hormonal products of the neoplasm, the age and sex of the patient, and perhaps upon other factors (1, 2). Because of the relative rarity of such lesions and the diversity of their manifestations, the reporting of a case which presents unusual features seems warranted.

In the case under consideration a benign adrenocortical tumor produced in a young woman the signs and symptoms of the adrenogenital syndrome. The urinary excretion of total and beta 17-ketosteroids was exceptionally high. Although menstruation had never occurred prior to removal of the tumor, menarche followed by pregnancy ensued after extirpation of the lesion. The breasts had failed to develop, however, and did not lactate following parturition.

CASE REPORT

A 28-year-old Brazilian woman had never menstruated and the breasts had not developed. At the age of 17 years she had observed a progressive growth of hair on limbs, face and abdomen and an increasing size of the clitoris. Three years before examination, the scalp hair became scarce. She was treated irregularly with small amounts of estrogens. During the preceding five years she had been undergoing electric depilation. She complained of uneasiness, irritability, tremor of the extremities and physical and psychic weariness.

Apparently the mother's pregnancy and parturition as well as the child's early growth and development had been normal. The patient suffered from frequent insomnia; sleep was light or agitated and accompanied by frightening dreams. There were no particular data concerning disturbances of behavior or sexual anomalies such as homosexuality. There was no difficulty in micturition or evacuation.

Physical examination: The patient presented a male appearance (Fig. 1). Her height was 161 cm. and her weight was 60 Kg. The blood pressure was 160/90 mm. of Hg. There was moderate hirsutism, baldness of the scalp (Fig. 2) and a conspicuous distribution of fatty tissue in the abdominal region. The musculature of limbs and trunk was of the male type. The face presented the effects of electric depilation. The skin was moist and the mucosae were of normal color. The sclerae were congested,

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* This study was made when the authors were members of the staff of Instituto Butantan, Sao Paulo, Brazil.

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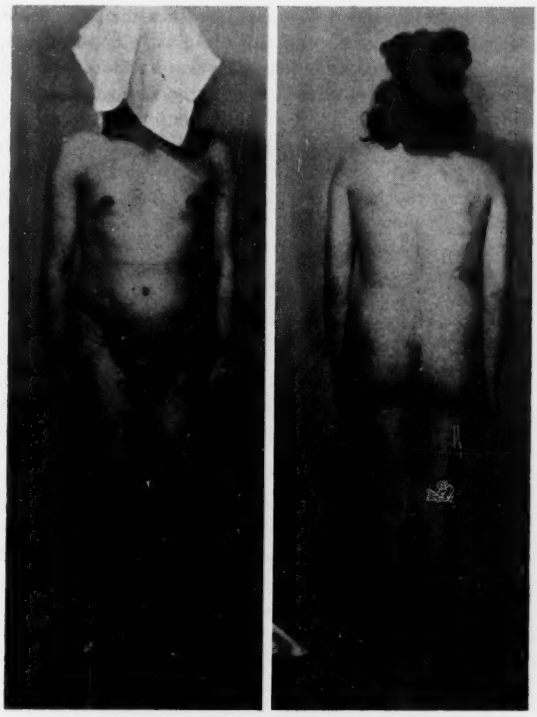

FIG. 1. Patient with body contour of the male type.

FIG. 2. Sparseness of hair in the central part of scalp (baldness).

the teeth were in good condition and there was a slight hyperemia of the nasopharynx. The voice was low-pitched. The thyroid was normal in size and consistency. The neck circumference was 30 cm. There was a small amount of fatty tissue in the breasts instead of true mammary glands.

Abdominal palpation suggested a subcostal tumor in the splenic region. The resistance

of the abdominal wall, however, did not permit identification of the spleen. The tumor was taken to be a deeply located mass communicating with the lumbar recess, similar to a hypertrophied kidney. There was an old surgical scar at the site of the clitoris.

Superficial reflexes were easily obtained. The extremities exhibited a coarse tremor. The patient was irritable and excitable. A definite inferiority complex was evident. She was greatly affected by the death of her father, the only person who knew about her pathologic condition.

Laboratory examinations: a) *Urinary 17-ketosteroids:* Several determinations by the method of Callow *et al.* (3) yielded an average value of 720 mg. per liter expressed as androsterone. A total volume of 11.6 liters of urine was collected and analyzed by Henriques, Henriques and Wendel (4). The neutral fraction, weighing 14.7 Gm., was

TABLE 1. INSULIN AND GLUCOSE TOLERANCE TESTS

Tests	Blood constituents	(milligrams per cent)						
		Fasting	minutes elapsed					
			20	30	60	90	120	150
Insulin (0.1 i.u./Kg.)	Glucose	94	49	57	68	78	77	—
	Phosphorus	3.1	2.8	2.6	2.3	2.6	2.6	—
Glucose ingestion	Glucose	97	122	119	128	125	98	90

treated with Girard's reagent to give 9.2 Gm. of ketonic material from which 1.8 Gm. of dehydroisoandrosterone and 0.6 Gm. of 3-chlorodehydroandrosterone were obtained. b) *Androgens:* The urine showed androgenic activity equivalent to 7.68 mg. per twenty-four hours of androsterone (chick comb weight method (5)). c) *Estrogens:* The biologic activity corresponded to 0.009 mg. of estrone per day (Burn's method (6)). d) *Gonadotropins:* The daily renal excretion amounted to as much as 100 m.u. (7). e) *Blood:* Examination revealed Hb 90 per cent, red cells 4,740,000 per cu. mm., white cells 5,500 per cu. mm.; 0 per cent banded, 38 per cent segmented neutrophils, 4 per cent eosinophils and 9 per cent monocytes. The sedimentation rate of erythrocytes was 3.5 mm. (Katz's index). The serum chloride content was 595 mg. per cent as NaCl (102 milliequivalents), and the cholesterol content was 134 mg. per cent. The results of insulin and glucose tolerance tests and variations in the serum phosphorus concentrations during the former test are given in Table 1. f) *Urine:* Absence of abnormal constituents.

X-ray examination: There were no abnormal shadows on the right side after perirenal insufflation. On the left side, however, the plates suggested a round tumor, uniformly shadowed on the periphery (Fig. 3). It occupied the whole renal area and encroached upon the lower edge of the spleen. The radiographic appearance was rather different from that usually seen in x-ray plates of adrenal tumors.

Summing up all these data, a diagnosis of a left adrenal tumor was made and operative treatment advised. During three days prior to the operation the patient received salt and desoxycorticosterone acetate. Sedatives and vitamins were also given.

Operation: Access to the left adrenal gland was obtained through Simon's incision (8) enlarged by resection of the twelfth rib. The latter was done because of the high position of the kidney. On top and in front of the superior renal pole a large gray tumor was found pressing on the spleen (Fig. 4). During the operation, which lasted one hour, the

FIG. 3. Perirenal insufflation (left). The arrows point to a tumor shadow surrounding the kidney. The spleen can be seen superimposed on the lower edge of the tumor.

patient received 10 cc. of adrenal cortex extract every fifteen minutes and 500 cc. of dextrose in saline once intravenously. Immediately after removal of the tumor the systolic blood pressure decreased progressively from 97 to 40 mm. of mercury, preventing exploration of the opposite side. Shock was overcome by means of plasma (250 cc.), blood (500 cc.), dextrose in saline (2 liters), 100 mg. of desoxycorticosterone acetate and 20 cc. of adrenal cortex extract.

FIG. 4. Adrenal tumor after its surgical removal (weight 735 Gm.)

Subsequent course: The postoperative period was uneventful. Administration of desoxycorticosterone acetate and adrenal cortex extract was discontinued after two days. The urinary excretion of 17-ketosteroids thirty days and two years after removal of the

tumor was 8.5 and 7.3 mg. per twenty-four hours, respectively. Menses appeared three months after operation, with scanty bleeding at first but increasing gradually until normal amount, duration and rhythmicity were attained. Thoracic and limb hair disappeared but the baldness of the scalp remained. Since the mammary glands remained undeveloped, estrogens were prescribed and the patient was kept under observation. Nervousness, irritability, depression and a slight brown pigmentation of the face and the back of the hands induced us to determine the basal metabolic rate, the amount of urinary 17-ketosteroids and the glucose tolerance, and to apply the Robinson-Power-Kepler test. The results of these tests were within normal limits. The Hanger test (cephalin), however, yielded a positive result (3 plus). Consequent upon a high intake of protein and carbohydrate, amino acid therapy and administration of *dl*-methionine and choline, the results of the Hanger test became only slightly positive (1 plus), coincident with partial disappearance of clinical symptoms.

FIG. 5. Cross-section of the tumor showing zones of necrosis.

The patient returned April 26, 1949, twenty-two months after removal of the tumor, presenting amenorrhea of four months' duration. A test for pregnancy gave a positive result. A normal parturition of a premature male child followed three months later. The boy was reared on an artificial diet and is in good condition at the present time.

Gross pathology: The tumor measured 153×125×100 mm. and weighed 735 Gm. It was covered by a thin fibrous layer, of greyish tint, and presented a smooth surface. On sectioning, central parts of the tumor showed irregular areas of hemorrhagic necrosis. At the periphery there were zones of a pale hue (Fig. 5).

Microscopic pathology: The neoplastic tissue of the compact areas was formed by polyhedral cells of different sizes with rich and homogenous protoplasm, and round and vesicular nuclei. These cells were spread in a fibrillar stroma with several blood vessels in their meshes. After scarlet staining, these cells showed the presence of fat droplets throughout the protoplasm. Some sections were filled with fat tissue in adipose alveoli. To summarize the pathologist's conclusion, the tumor was formed mainly at the expense of the fascicular layer of the adrenal cortex and none of the cells displayed malignant characteristics (Fig. 6, a and b).

COMMENT

This case is instructive, first of all, because of the enormous excretion of 17-ketosteroids, most of which was dehydroisoandrosterone. This was

taken to be a sign of malignancy, but microscopic examination of the tumor and its failure to recur during a period of approximately two years following its removal tended to contradict this assumption. The value of 720 mg. of 17-ketosteroids per liter expressed as androsterone, is among the highest registered in the literature (9). Kepler *et al.* (10) observed 3 cases with excretions of 800, 857 and 1,005 mg. per twenty-four hours, respectively. In these 3 cases, as well as in ours, the increase was largely in the beta fraction of the ketonic material. This fact confirms the conclusion that a beta fraction which is 50 per cent or more of the total, indicates the presence of a tumor. On the other hand, the low excretion of estrogens (0.009 mg. of estrone per twenty-four hrs.) is in agreement with Frank's

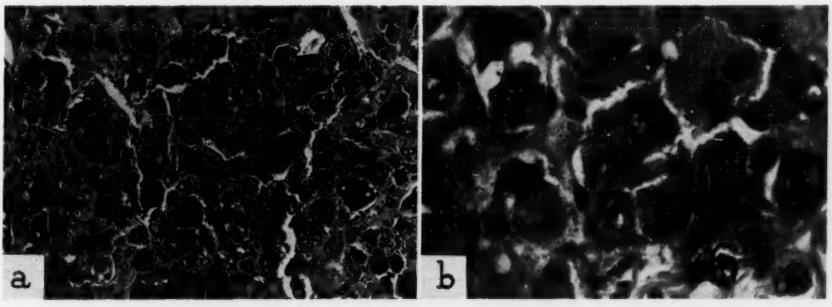

FIG. 6. Microscopic aspect of a well-preserved portion of the tumor to show the conspicuous cells with abundant protoplasm and vesicular nuclei; (a) ×112, (b) ×650.

opinion (11) that benign cortical tumors are accompanied by a low urinary output of estrogenic substances.

Noteworthy also in this case was the rapid return of the ovaries to normal function after removal of the tumor. Amenorrheic for fifteen years, the patient started rhythmic menstrual flow three months after the operation and became pregnant fifteen months later. Although the parturition was normal, lactation failed completely.

Review of the literature at our disposal revealed that reports of 19 cases of adrenocortical tumor which were successfully operated on have appeared since 1937: 9 cases were reported by Walters (12), 3 by Reilly *et al.* (13, 14), 1 by Cahill *et al.* (15), 3 by Mintz and Geist (16), 1 by Soffer (2), 2 by Wolfson *et al.* (17) and 1 by McCullagh (18). Recently Walters and Sprague (19) pointed out the importance of adequate preoperative and postoperative measures for the success of the surgical treatment. Indeed, postoperative adrenal insufficiency, if not adequately treated, may lead to a fatal end in some cases.

SUMMARY

An instance of benign adrenal cortical tumor is reported. The tumor was associated with a high excretion of 17-ketosteroids (720 mg. per liter), a large proportion of which was dehydroisoandrosterone.

The patient, a 28-year-old woman who had never menstruated, had her first menstrual flow three months after removal of the tumor and became pregnant fifteen months later. Although parturition was normal, lactation failed completely.

Acknowledgment

Acknowledgment is due to our colleagues J. R. Valle, E. Costa Manso, Camilo G. Almeida, J. R. Meyer, H. Sampaio Correia, J. Kubo and Francisco R. Nobre, whose help was truly appreciated. The authors are also indebted to Laborterapica, S. A. for the liberal supply of adrenal cortex extract.

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The Association for the Study of Internal Secretions

THE 1951 ANNUAL MEETING

The Thirty-third Annual Meeting of the Association for the Study of Internal Secretions will be held at the Chalfonte-Haddon Hall Hotel, Atlantic City, New Jersey. The Scientific Sessions have been extended to three days, Thursday, Friday, and Saturday, June 7-8-9.

The Local Chairman of Arrangements is Dr. Matthew Molitch.

Due to the advance in the date of our meeting, Chalfonte-Haddon Hall Hotel will be unable to accommodate all of our members by reason of a previous commitment. The Colton Manor, on Pennsylvania Avenue near Haddon Hall, is an excellent hotel, which has been recently refurnished, and is holding one hundred rooms for our members on the "first come, first served" basis. The Hotel Strand, at Pennsylvania Avenue and the Boardwalk, has also promised to supply a limited number of rooms. It is important that reservations be made immediately if you are to avoid disappointment.

All Scientific Sessions will be held in the Chalfonte-Haddon Hall Hotel. The rooms, in which the session will be held, will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, June 8, at 7:30 P.M. in the Rutland Room, preceded by cocktails at 6:30 P.M. in the adjoining Benjamin West Room.

Those wishing to present papers, which will be limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. Jane Russell, School of Medicine, Emory University, Atlanta, Georgia, not later than *March 1, 1951. It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and journals of the Association.* The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.

2. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each non-member author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of non-members who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.
   Line 3. Institution of origin and city in which institution is located.
3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
4. Abstracts should be letter-perfect, since there will be no opportunity for proof reading by authors.

(For details about the type of program, *see* Dr. E. H. Rynearson's Letter to the Editor on page 1355 of the October 1950 issue, and Dr. Jane A. Russell's Letter to the Editor on page 229 of the February 1951 issue.)

THE 1951 AWARDS AND FELLOWSHIPS

Fellowships

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship will not be awarded in 1951, so that it may accumulate toward a $5,000.00 Fellowship to be awarded in 1952.

THE SCHERING FELLOWSHIP IN ENDOCRINOLOGY

The Schering Fellowship in Endocrinology was established in 1949 and the first recipient was Dr. D. Laurence Wilson, who also received the 1950 Fellowship.

Association Fellowships are designed to assist men or women of exceptional promise in their progress toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D. or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $2,500.00, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation

of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

Awards

THE E. R. SQUIBB AND SONS AWARD

The E. R. Squibb & Sons Award of $1,000.00 was established in 1929, and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr E. A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; in 1949 to Dr. Herbert M. Evans; and in 1950 to Dr. C. N. H. Long. In 1943 no award was given. A special committee of five members of the Association selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

THE CIBA AWARD

The Ciba Award to recognize the meritorious accomplishment of an investigator not more than thirty-five years of age in the field of clinical or preclinical endocrinology was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; in 1949 to Dr. George Sayers; and in 1950 to Dr. Oscar M. Hechter. The Award is for $1,200.00. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $1,800.00.

NOMINATIONS

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in, endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on special application forms which may be obtained from the *Secretary, Dr. Henry H. Turner,* 1200 North Walker Street, Oklahoma City 3, Oklahoma, and returned to him *not later than March 1, 1951.*

FOURTH ANNUAL POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY INCLUDING DIABETES

Sponsored by

THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

and

THE AMERICAN DIABETES ASSOCIATION

SEATTLE, WASHINGTON OLYMPIC HOTEL JULY 2–7, 1951

The faculty will consist of prominent researchers and clinicians in the field of endocrinology and metabolic disorders.

The course will be a practical one of interest and value to the specialist and those in general practice. The program will consist of lectures, clinics, and demonstrations. Ample time will be given to questions and answers at the end of each session, and registrants are encouraged to contact members of the faculty for individual discussions.

The Olympic, one of Seattle's most delightful hotels, offers special convention rates to members of this assembly. This is an unusual opportunity for you and your family to enjoy a pleasant vacation in the beautiful Pacific Northwest and for you to participate in a highly instructive program of the latest advances in endocrinology and metabolism.

A fee of $75 will be charged for the entire course and the attendance will be limited to 100. REGISTRATION WILL BE IN THE ORDER OF CHECKS RECEIVED AND WILL CLOSE ON JUNE 4, 1951. SHOULD THERE BE AN INSUFFICIENT NUMBER OF APPLICANTS TO FILL THE COURSE, THE REGISTRATION FEE WILL BE REFUNDED IMMEDIATELY IN ITS FULL AMOUNT.

Please forward application on your letterhead, together with check payable to The Association for the Study of Internal Secretions, to Henry H. Turner, M.D., Secretary-Treasurer, 1200 North Walker Street, Oklahoma City 3, Oklahoma, *before June 4, 1951*. Further information and program will be furnished upon request.

Hotel reservations should be made directly with the Olympic Hotel, Seattle, Washington, and the hotel advised that you are attending this Postgraduate Assembly.

The American Goiter Association

THE 1951 ANNUAL MEETING

THE 1951 annual meeting of the American Goiter Association will be held in Columbus, Ohio, on May 24, 25, and 26. The Deshler-Wallick Hotel, Broad and High Streets, will be the headquarters and every effort will be made by the hotel officials to make the meeting a success and the visit a very pleasant one. It would be well to see that reservations are made now.

The president, Dr. T. C. Davison, and the chairman of the Local Arrangements Committee, Dr. George Curtis, are planning an excellent meeting which will consist of papers dealing with goiter and other diseases of the thyroid gland, dry clinics and demonstrations. It is hoped that there will be a large attendance of members.

GEORGE C. SHIVERS, M.D.,
Corresponding Secretary,
Colorado Springs, Colo.

VAN METER PRIZE AWARD FOR 1951

THE American Goiter Association again offers the Van Meter Prize Award of three hundred dollars, and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association which will be held in Columbus, Ohio, May 24, 25, 26, 1951, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double spaced copy in duplicate sent to the Corresponding Secretary, Dr. George C. Shivers, 100 East Saint Vrain Street, Colorado Springs, Colorado, *not later than March 1, 1951.* The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award Essay by the author, if it is possible for him to attend. The essay will be published in the annual Transactions of the Association.